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Cesar Rigoberto Molina

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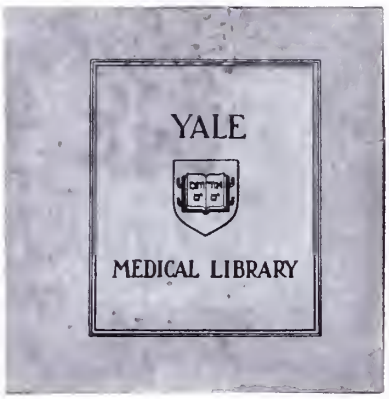
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INTERORGAN METABOLISM IN THE POSTABSORPTIVE STATE AND AFTER A BRIEF FAST IN AWAKE DOGS

Cesar Rigoberto Molina

"A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Medicine Yale University School of Medicine"

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The Vedas' concern is with the three
gunas. Be without the three gunas,
O Arjuna, freed from duality, ever
firm in purity, independent of possessions,
possessed of the Self.

This is the technique of instantaneous realization.

Maharishi
Mahesh Yogi

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Introduction

Starvation is rarely a social problem encountered by American and European peacetime medicine. The recent interest in the physiology of starvation lies in the fact that starvation is a significant factor in the morbidity and mortality of patients with severe uncontrolled diabetes mellitus, metastatic cancer, inflammatory bowel disease, trauma, uremia, and sepsis. These disorders are characterized by inadequate intake and the absence of the normal homeostatic response to food deprivation characterized by the sparing of protein and the oxidation of fat (Cahill, 1966). These patients die due to the depletion of muscle protein, which results in weakness of the respiratory muscles (with decreased tidal volumes and cough reflex), followed by 1) the accumulation of secretions and 2) atelactasis leading to pneumonia and death. Garrow (1965) estimates that man can only spare 30-50% of his total body protein before death ensues.

The interest in the metabolic and biochemical response to food deprivation is not new. In 1912, Dr. Francis Benedict, in a well designed and carefully conducted study, fasted a normal man for 30 days. By measuring metabolic rate, respiratory quotient and urinary nitrogen excretion, he described a progressive decrease in protein catabolism, as manifested by decreased urinary nitrogen excretion and a reliance on adipose tissue for fuel (1915). In that same year Dr. P. E. Howe fasted a dog for 117 days. The dog tolerated the fast well, losing about 16 kg. of body weight (Howe, 1912). Dr. Howe documented a fall in urinary nitrogen excretion. This dog excreted an average of 6.23 grams of urinary nitrogen per day during the first 4 days of the fast as compared to an average of 2.44 grams/day during the last 4 days of the fast.

The elegant experiments of Cahill, Pozefsky, Owen, Felig and Aoki during the past 2 decades have lead to a better understanding of the metabolic changes which occur in fasting man. The use of regional bed (muscle, liver, kidney, brain) catheterization studies and isotope tracer techniques by these investigators revealed the role of different organs in overall fuel metabolism in man (Owen, 1969, Aoki, 1976, Felig, 1969). According to these investigators the endocrine and metabolic response to food deprivation is a carefully orchestrated hormone-fuel-tissue interplay designed to provide the tissues, particularly the brain, with their fuel requirements, while at the same time sparing protein, and consuming fat (Cahill, 1970).

It is technically difficult to study the metabolic interplay of the major organs of man during different periods of a fast, particularly with regard to monitoring the non-hepatic splanchnic bed. Additional problems include the difficulty of restudying the same volunteers on different diets as well as the difficulty in obtaining F.D.A. and Human Studies Committee approval for the infusion of a variety of substances. Consequently the need for a new model has arisen. The sheep has been extensively studied by Bergman (1978) and Shoemaker and Katz (1966) but since it is a ruminant and the absorbed substrates are the product of fermentation rather than of digestion it is difficult to make extrapolations to human physiology. The rat has also been extensively studied but its small size and blood volume make the in vivo study of interorgan metabolism difficult (Blackshear, 1974, Ruderman, 1977). Klain (1977) recently studied the effects of starvation on the plasma level of many metabolites in the pig. We have tried to develop a suitable model to study the interorgan metabolism of this animal, but we found it very difficult with which to work. We have therefore chosen the dog whose relatively large size, blood volume, and tractable nature makes it an

almost ideal model to study hormone-fuel interrelationships. The purpose of this thesis is to demonstrate the similarities and differences between the human and the dog models when they are briefly starved. Particular attention has been placed on the utilization and production of glucose. In order to understand the complex interorgan hormone-fuel interrelationships modulating carbohydrate and protein metabolism during a brief fast we have obtained simultaneous arterial and venous samples across the liver, non-hepatic splanchnic bed, kidney and hindleg of conscious dogs with multiple pre-implanted catheters.

In man the adaptation to food deprivation is usually viewed as a continuum (Cahill, 1975) composed of a sequence of metabolic alterations wnich are divided into:

1) The completion of the gastrointestinal absorption of substrates which, depending on the amount and quality of the meal ingested, occurs approximately six hours after the last meal. This phase will not be discussed in this thesis.
2) The postabsorptive phase or glycogenolytic phase which is usually temporally defined by an overnight fast or 12-18 hours after the last meal.
3) The gluconeogenic phase as defined by a brief fast usually lasting from 60 to 72 hours after the last meal.
4) The protein sparing phase in which protein is maximally conserved and the brain's requirements for glucose are replaced by the products of fat mobilization and oxidation (Owen, 1967).

The above mentioned temporal phases in the human metabolic adaptation to food deprivation have as their main purpose the derivation of energy from adipose tissue and the sparing of protein by reducing the terminal

oxidation of glucose and therefore the rate of gluconeogenesis from amino acids.

The Postabsorptive State

After an overnight fast, the organism continues to require energy which has to be mobilized from endogenous stores. Man and dog have two major fuel depots, adipose tissue and muscle protein (Cahill, 1970, Romsos, 1976). Carbohydrate is stored as glycogen in the liver and muscle but it serves only as a minor energy depot, maintaining euglycemia for less than 24 hours (Nilsson, 1973). Protein's main purpose is not the storage of energy. Every protein molecule appears to have a function, whether it be an enzyme, a structural component of tissues as in collagen and elastin or a contractile molecule as in myocardium and skeletal muscle. Every effort is made to spare protein from becoming a source of energy, since it makes up the machinery (Ruderman, 1975). Fat's main function is to serve as a fuel depot, making up 20% of the mass of the individual but storing 80% of the energy (Saudek, 1976). Romsos, using a tracer technique, calculated the body fat content of 9 month old beagles to be 21-30% of the total body mass (1976). These values are comparable with those quoted for man.

The first adaptation to the lack of exogenous calories is the return of insulin concentrations to basal levels leading to the exclusive oxidation of free fatty acids, with the resultant exclusion of glucose from the majority of tissues, particularly muscle, kidney, and liver. As a result, the respiratory quotient of the fasting individual approaches .7 (Owen, 1967). Glucose catabolism continues in non-insulin dependent tissues; the nervous system, the renal medulla and the cellular element of the blood and bone marrow. Glucose is utilized at a rate of 2-3 mg/kg of body weight per minute in both dog and human (Cowan, 1969, Jennings, 1977, Ruderman,

1976, Felig, 1976). The amount of glucose utilized in the postabsorptive state depends in part on the amount of carbohydrate consumed the night before (Cowan, 1969). However, after 48-60 hours of food deprivation the relative composition of the preceeding diet has no effect on glucose utilization provided that enough protein is included in the diet (Cowan, 1969). In order to maintain euglycemia the organism must balance glucose production and consumption or must decrease the terminal oxidation of glucose. After an overnight fast, a brief 2-3 day fast, and a prolonged fast, both mechanisms are in operation but have different importance in the maintenance of euglycemia (Cahill, 1975).

Glycogen is an important source of glucose during the first 24 hours of a fast (Nilsson, 1973). Seventy five percent of the glucose produced after an overnight fast in man arises from liver glycogen (Felig, 1979). Liver glycogenolysis is accelerated by falling insulin levels and increasing glucagon concentration (Chiasson, 1974). The other 25% of the glucose produced and released by the liver in the postabsorptive state is the product of gluconeogenesis (Chiasson, 1977).

Gluconeogenesis is the production of glucose from such precursors as lactate, pyruvate, glycerol, and amino acids except leucine. Gluconeogenesis is the result of a carefully orchestrated interchange of these metabolites between the adipose tissue, glycolytic tissues, muscle and visera. The production of new glucose is an energy consuming reaction. This energy is provided by the hepatic oxidation of free fatty acids (FFA) which are released from adipose tissue. The liver is the main gluconeogenic organ in man during a brief fast and is the center of the metabolic transformation as the organism adapts to food deprivation (Cahill, 1970). Felig (1968), by infusing gluconeogenic precursors to fasting individuals has shown that the liver's rate of gluconeogenesis depends upon substrate

availability as well as the hormonal milieu. Therefore, the hepatic adaptation to food deprivation is dependent in part on the metabolic alterations which occur at the periphery.

Adipose tissue supplies both glycerol and free fatty acids, the main source of energy for the fasting individual. They are either terminally oxidized to CO_2, recycled back to triglycerides or converted to ketones (Wiener, 1971). After an overnight fast the concentration of free fatty acids in man is twofold higher than 2 hours after a meal (Felig, 1979). Postabsorptive man mobilizes about 7 grams of FFA per hour. Of these one third are terminally oxidized; a third is recycled to triglycerides and the rest is converted to ketone bodies, β-hydroxybutyrate (BOHB) and acetoacetate (AcAc).

Basso (1970), using labeled palmitate and arteriovenous concentration differences across the liver, non-hepatic splanchnic bed and hindleg of the 18 hour fasted anesthesized dog studied the metabolism of free fatty acids. The net production of FFA is 6.31 μmoles/min/kg of body weight. The liver extracts 25% of the free fatty acids presented to it and does not produce FFA. The non-hepatic splanchnic bed extracts free fatty acids but also produces them, accounting for 3-20% of the total net release of free fatty acids extracted by the liver. Of the free fatty acids extracted by the liver, 13% are secreted as triglycerides, only 10% are terminally oxidized to CO_2 and 12-24% are converted and released as ketone bodies. Ketone body production is 1.03 μmoles/kg/min. They are an important substrate. Canine myocardium can derive up to 80% of its energy from ketones (Little, 1970). Their role in the postabsorptive state is unknown. In addition, the non-hepatic splanchnic bed is known not to extract ketones after an overnight fast (Basso, 1970).

Ketogenesis and fat mobilization are hormone sensitive reactions. Insulin appears to be the main regulator (Keller, 1977). Although the

availability of FFA is important, the falling level of insulin is the predominant regulatory factor (Keller, 1977). Keller demonstrated that increasing the FFA supply per se was associated with only limited stimulation of ketogenesis whereas the combination of insulin deficiency, basal concentration of glucagon, and increased FFA levels produced a synergistic augmentation of hepatic ketone production (Keller, 1977). The falling level of insulin enhances the activity of the mitochondrial acylcarnitine transferase system which leads to increased ketone production (Felig, 1979, Keller, 1978). Basal insulin levels are also responsible for increased fat mobilization, permitting the hormone sensitive lipase to catalyze the mobilization of triglycerides (Fine, 1960). Therefore, there is increased FFA and glycerol availability.

Ketone bodies are known to play an important role in protein metabolism. The addition of acetoacetate to the rat diaphragm in vitro by Palaiologos and Felig (1976) lead to decreased alanine production and decreased proteolysis. AcAc was found by these investigators to also inhibit glycolysis. Studies in vivo by Sherwin (1975) have demonstrated that an infusion of β-hydroxybutyrate in postabsorptive man leads both to a fall in plasma glucose, alanine and urinary nitrogen excretion. Reichard (1974) found that ketone body oxidation falls as a fast progresses. Decreased ketone oxidation appears to be the basis of the ketonemia seen in fasting man since insulin concentrations and hepatic ketone production plateaus after 3 days of starvation (Owen, 1971, 1973).

Ketonemia is a prerequisite for significant human brain ketone oxidation. With prolonged fasting these levels are high enough so as to cross the blood brain barrier and replace glucose as the main fuel for the central nervous system (Owen, 1967). This permits protein sparing since less glucose is terminally oxidized by the brain. Ketones are

produced at a similar rate by the dog but the ketonemia associated with prolonged fasting in this species is insignificant when compared to man. It is apparent that the role of ketones in the adaptation to food deprivation is different in man and dog.

Glycerol, another product of fat mobilization, plays a significant role in glucose homeostasis in both dog and man. Two percent of human splanchnic output of glucose after an overnight fast is produced from glycerol (Felig, 1976). Glycerol's role in gluconeogenesis in the dog is more important than in man; after an overnight fast 6% of splanchnic gluconeogenesis is derived from glycerol (Hall, 1976). Approximately 60-70% of circulating glycerol is extracted by the liver of the dog while 20% is retained by the non-hepatic splanchnic bed (Basso, 1970). The glycerol thus extracted by the non-hepatic splanchnic bed can contribute to triglyceride synthesis since there is a glycerokinase in the canine intestine and there is no glucose production by this tissue (Basso, 1970)

Lactate and pyruvate are very important gluconeogenic substrates in both man and dog. Muscle glycogen accounts for two times more glucose than the liver glycogen stores (Nilsson, 1973, Cahill, 1970). Since muscle does not possess glucose-6-phosphatase it cannot break down glycogen to glucose (Stryer, 1976). The glucose-6-phosphate provided by muscle glycogenolysis has to undergo glycolysis in order to enter the circulation. The pyruvate produced from glycolysis is either released directly into the circulation, converted to lactate and enters the Cori-cycle or is transaminated to alanine and becomes part of the alanine-glucose cycle (Felig, 1973). The pyruvate released into the circulation accounts for only 1% of hepatic gluconeogenesis while lactate accounts for 10-15% (Felig, 1979). Glucose produced from lactate is metabolized by the

glycolytic tissues of the body which again produce lactate. This is known as the Cori-cycle whose purpose is to shuttle, in the form of glucose, energy from the liver (derived from ketogenesis) to glycolytic tissues (Cahill, 1970).

In the postabsorptive state a significant amount of hepatic gluconeogenesis arises from alanine generated by mobilization of muscle glycogen stores (Chiasson, 1977). Alanine accounts for 10% of splanchnic glucose production. Fifty percent of that alanine arises from muscle (Felig, 1973b). Muscle protein is 7% alanine by composition but alanine constitutes 30% of the amino efflux from muscle (Ruderman, 1975). This discrepancy is due to the de novo synthesis of alanine by the transamination of pyruvate (Goldberg, 1978, Odessey, 1974). Alanine serves a dual purpose. It transfers the pyruvate generated from muscle glycogenolysis and glycolysis and the amino groups generated from the transamination and oxidation of the branched chain amino acids (Goldberg, 1978). Therefore, alanine synthesis permits the transfer of pyruvate from muscle to liver for reconstitution back to glucose and the oxidation of the branched chain amino acids without the accumulation of ammonia. This is an energy generating reaction. Alanine production from glucose yields 8 moles of ATP as compared to 2 ATP moles generated if lactate is instead produced. Furthermore, the complete oxidation of the branched chain amino acids (BCAA) produces 44 moles of ATP. Recently Aoki (1980) has challenged the role of alanine as the most important carrier of BCCA derived amino groups from muscle. He found that after a leucine meal glutamine efflux increased 3 fold and after exercising the forearm, glutamine efflux increased 8 fold with only a modest change in alanine output.

The alanine released by muscle is avidly extracted by the postabsorptive liver and reconstituted back to glucose. The amino group

goes into urea production. The new glucose produced from muscle derived alanine can be recycled back into alanine and serve as an energy shuttle from liver to periphery similar to the Cori cycle. The recycling of glucose through alanine is known as the glucose-alanine cycle and was first described by Mallette (1969) and Felig (1973a).

The other 50% of the alanine extracted by the human and dog liver is produced, not by muscle, but by the non-hepatic splanchnic bed (Elwyn, 1968, Weber, 1977, Matsukata, 1973, Vaidyanath, 1978, Felig, 1973b, Aikawa, 1973). Glutamine, (which like alanine constitutes only 7% of the skeletal muscle protein and accounts for 23% of the amino acid efflux) could possibly contribute its carbon skeleton and nitrogens for the alanine released from the gut (Matsutaka, 1973). This close relationship between muscle, gut and liver has been observed in man, dog and rat (Windmuller, 1975). The glutamine extracted is ultimately produced by the Krebs cycle via transamination reactions from α-ketoglutarate to glutamate to glutamine (Chang, 1978b).

Goldberg and Chang (1978) working on the rat diaphragm in vitro have demonstrated that the carbon skeletons for glutamine synthesis arise from the deamination of valine, glutamate, aspartate and isoleucine. These amino acids and leucine are the only ones oxidized by skeletal muscle. The glutamine-derived alanine from the gut leads to net glucose production unlike the muscle derived alanine which is part of the glucose-alanine cycle.

Alanine and glutamine are not the only important amino acids involved in the interchange of energy from muscle to splanchnic bed. The branched chain amino acids (BCAA) particularly leucine (the only non glucogenic amino acid) play a very important role in protein metabolism. BCAA are preferentially oxidized at the periphery and the brain (Felig, 1973c). Studies on rat heart and diaphragm in vitro as well as infusions of

leucine in vivo strongly suggest that leucine metabolism plays an important regulatory effect on muscle anabolism and catabolism. Buse (1975) and Fulkes (1975) found that the addition of leucine into the media stimulated the synthesis of muscle protein in vitro. Sherwin (1978) found that an infusion of leucine will lead to decreased urinary nitrogen excretion, decreasing gluconeogenesis in postabsorptive, briefly and prolonged fasted subjects. The oxidation of the BCAA appears to be rate limiting in protein degradation (Aoki, 1978). Since the initial oxidation reaction occurs in the mitochondria and requires NAD, Aoki believes that the mitochondrial redox state (NADH/NAD) may be a determining factor in their rate of oxidation. The oxidation of the BCAA α-keto-analogs requires NAD and is inhibited by NADH in a manner similar to pyruvate and α-ketoglutarate dehydrogenase (Aoki, 1978). In summary, the oxidation of the BCAA and the levels of ketone bodies are important controlling factors in muscle proteolysis which is the source of gluconeogenic substrates. Therefore, they are very important in glucose and protein metabolism since liver, the central gluconeogenic organ, is dependent on the gluconeogenic substrates released by muscle. Thus it is believed that BCAA and ketones are important in producing protein sparing.

Lactate and alanine are the main, but not the only, gluconeogenic substrates. Other amino acids are also involved in glucose production. These arise from the breakdown of liver and muscle protein. In the postabsorptive state, muscle is in net negative nitrogen balance. Most amino acids are released, as demonstrated by arterio-venous differences across the forearm and leg, except for small uptakes of serine, cystine and glutamate (Aoki, 1973, Marliss, 1971, Felig, 1970, Pozefsky, 1969). A similar pattern of amino acid balances across skeletal muscle has been

observed in the rat and sheep (Aikawa, 1973, Bergman, 1978). Arteriovenous differences across the dog hindleg during postabsorption have not been measured.

The consistent uptake of amino acids across the splanchnic bed complements the negative balance of amino acids across muscle (Marliss, 1971, Aoki, 1976, Felig, 1969, 1975). As noted previously, the splanchnic bed measurements represent the net balance between the interactions of the non-hepatic splanchnic bed (NHSB) and the liver. In addition to the interesting interaction of alanine and glutamine across the two components of the splanchnic bed and skeletal muscle, other amino acids are preferentially handled by the NHSB and liver. Felig and Wahren, in studies during elective surgery in anesthesized postabsorptive humans, demonstrated a negative amino acid balance across the NHSB (Felig, 1973b). Taurine, threonine, citrulline, glycine, alanine, valine, methionine, leucine and phenylalanine were all released by the portal vein drained viscera. There was an uptake of glutamine, the only amino acid significantly extracted. However, in the dog, Weber (1977) found that amino acids other than glutamine were extracted; small amounts of serine, leucine, valine, arginine, isoleucine, phenylalanine, tyrosine and histidine were extracted and only alanine, proline and citrulline were released. Weber conducted his studies on 24 hour fasted anesthesized mongrel dogs. He selectively studied the jejunum rather than the total portal drained viscera (small intestine, part of colon, stomach, spleen, pancreas, omentum) studied by Felig. In addition to alanine extraction by the liver, serine, threonine and glycine are significantly removed as well as most of the other glycogenic amino acids. A notable exception are the branched chain amino acids which are not usually extracted by the liver (Felig, 1975, Aikawa, 1973, Elwyn, 1968). The liver, with the

exception of the BCAA, will extract what the NHSB and skeletal muscle produce.

The kidney is significantly involved in fuel metabolism. In the postabsorptive state, the human kidney minimally consumes glucose and its calculated energy requirements are provided by the oxidation of free fatty acids (50%), citrate (10%) and lactate (35%) (Cahill, 1975, Neith, 1964). The kidney, unlike liver and muscle, has very limited glycogen reserves so that renal glycogenolysis plays an insignificant role in glucose homeostasis in the overnight fasted individual (Cahill, 1975). Net balance studies done by Owen and Robinson (1963) in postabsorptive non-obese subjects showed an uptake of glutamine, proline, and glycine. The kidney produces serine, alanine and ammonia. The kidney is therefore the source of the serine which is extracted by both the splanchnic bed and skeletal muscle. The alanine released by the kidney is of small quantity (10-20 μmole/min) as compared to muscle which releases about 100 μmole/min/100 cc of forearm (Owen, 1963, Pozefsky, 1969).

Churchill (1970), in both awake and anesthesized postabsorptive dogs did not observe any production of glucose by the kidney. In fact he observed a small uptake of glucose (.765 ± .292 μmoles/kg/min). Dies (1970) did not observe any net balance of glucose across the kidney but observed a net uptake of FFA, lactate and citrate. Their findings contradict those previously reported by Cohn (1951) who showed a net renal glucose production of 60 mg/kg of body wt./hour or "one fourth of the basal glucose requirement." Shalhoub (1963) measured amino acid arterio renal vein differences in anesthesized dogs under different acid base conditions. He reported a similar pattern of amino acid balances in the dog as compared to man.

The human as well as dog brain oxidize a considerable fraction of

the total glucose consumed (Cahill, 1970). After an overnight fast most of the tissues use free fatty acids, but not the brain which cannot oxidize them. The human brain oxidizes about 144 grams of glucose per day (Cahill, 1970b). Since most of this glucose is terminally oxidized and not recycled like in the other non-insulin dependent glycolytic tissues it has to be replaced in order to maintain euglycemia (Cahill, 1966). In order to meet this demand the organism is forced to change its metabolic switch from fed to fasting. This switch from fed to fasting is directed at providing the brain with an alternate source of fuel thereby sparing protein derived gluconeogenesis (Owen, 1967). The postabsorptive man and dog brain continues to oxidize glucose along with other amino acids. Felig measured arterio venous differences across the brain in postabsorptive normal subjects (Felig, 1973). He demonstrated an uptake of most amino acids. The uptake of valine exceeds that of all other amino acids. Thus, the brain is important in the disposal of the branched chain amino acids which are released by skeletal muscle and are not extracted by the viscera.

Glucoregulatory Hormones

Glycogenolysis, gluconeogenesis, lypolysis and ketogenesis are regulated by both substrate availability and the hormonal milieu. In the postabsorptive state and during a brief fast the falling insulin and rising glucagon levels are the most important modulating factors. The importance of insulin is suggested by the inhibition of glycogenolysis, gluconeogenesis, lypolysis and ketogenesis during the intestinal absorptive phase when their respective precursors are abundant but insulin concentrations are elevated (Aoki, 1979, Chiasson, 1976). In man the initial response to food deprivation is regulated by the falling insulin levels accompanied by rising glucagon concentrations

(Cahill, 1966).

Insulin

Insulin returns to basal levels 12-18 hours after the last meal. Basal insulin concentrations are 10-20 μU/ml in both dog and man (Cherrington, 1976). Insulin secretion does not cease after an overnight fast. Cherrington (1976) had to infuse 400 μU/kg of body weight/min into the portal vein in order to maintain basal arterial insulin levels in somatostatin treated dogs.

Studies in vitro and in vivo have demonstrated that insulin plays an important role in amino acid metabolism in skeletal muscle. Incubation of rat diaphragm in vitro in the presence of glucose and insulin decreased proteolysis and stimulated protein synthesis (Fulks, 1975). Pozefsky (1969), in vivo, showed that insulin infusions into the forearm of man decreases amino acid efflux. Finley (1980) found that 75 minutes after forearm exercise and insulin infusion there is a net uptake of α-amino acids by the fasted forearm muscle. Therefore, falling insulin levels will lead to increased muscle proteolysis with increased substrate availability for gluconeogenesis.

Adipose tissue is very sensitive to the effects of insulin in the postabsorptive state (Aoki, 1979). Falling insulin concentrations will decrease the activity of the hormone sensitive lipase leading to enhanced triglyceride mobilization (Harper, 1976). Fat mobilization will also enhance glycerol release which accounts for 2% of human and 6% of dog splanchnic gluconeogenesis (Felig, 1979, Hall, 1977).

Acylcarnitine transferase activity increases as the suppressive levels of insulin return to basal levels (Keller, 1978). This is a very hormone sensitive enzyme since ketogenesis will be inhibited with small glucose meals in prolonged fasted man without affecting the mobilization

of triglycerides and the concentrations of free fatty acids (Aoki, 1975b).

The main effect of insulin is at the liver. Cherrington (1976), working on the dog model, showed that insulin prevents the glucagon-enhanced hepatic glucose production. He showed that euglycemia is attained by a balance between the stimulating effect of glucagon and the inhibitory action of insulin. Chiasson (1976) observed in dogs that glycogenolysis is more sensitive than gluconeogenesis to the inhibitory effects of small changes in insulin concentration. This explains in part why glycogenolysis is dominant in glucose production during the early part of a fast. Glucose splanchnic production has been shown to be dependent not on the absolute levels of insulin and glucagon, but on their ratio (Parrilla, 1974). After an overnight fast the insulin: glucagon ratio decreases thereby favoring glucose production.

Glucagon

Normal glucose homeostasis in the postabsorptive state is dependent in part on glucagon concentrations. This is true in both dog and man. Alford (1974) infused somatostatin, a tetradecapeptide which inhibits pancreatic insulin and glucagon secretion, into postabsorptive man and found a drop in blood glucose. Glucose levels tend to return to normal or even higher concentrations afterwards, since the concominant lack of insulin decreases the peripheral utilization of glucose. Cherrington (1976), by infusing somatostatin showed that at least one third of basal glucose production is dependent upon basal glucagon levels. Glucagon enhances hepatic glucose production by both stimulating glycogenolysis via adenyl cyclase and by increasing alanine derived gluconeogenesis (Chiasson, 1974).

Short Term Starvation

The response to food deprivation becomes more pronounced after a

brief fast (3-4 days). There is an acceleration of gluconeogenesis, lypolysis and ketogenesis, processes which were already active after an overnight fast. Gluconeogenesis is enhanced by both the continual requirement for glucose, particularly the brain, and the depletion of glycogen stores (Garber, 1974).

The increased rate of gluconeogenesis after a brief fast is mediated by the falling levels of both glucose and insulin and the concomitant rise in plasma glucagon. Insulin and glucose concentrations fall until the third day of a fast when they plateau (Cahill, 1966). The fall in insulin levels and the increased glucagon concentration lead to both enhanced extraction of gluconeogenic substrates and increased gluconeogenesis. Amino acids and glycerol are the main gluconeogenic substrates leading to net glucose production (Aoki, 1975a). An increased rate of gluconeogenesis then requires an increased rate of lypolysis and proteolysis to provide both the glycerol, amino acids and the energy required for splanchnic glucose production.

Pozefsky (1976) studied amino acid fluxes across the forearm of 15 postabsorptive subjects and compared them to values obtained in another group of seven subjects who had fasted for 60 hours. He demonstrated a 70% increment in the release of the principal glycogenic amino acids. Alanine release increased by 60% and there was a significant increment for threonine, serine, glycine, α-aminobutyrate, methionine, tyrosine and lysine. Finley (1980), working at Cahill and Aoki's laboratory, demonstrated an increment in muscle proteolysis after a 60 hour fast assessed by an elevated urinary nitrogen and 3-methylhistidine excretion. However, he did not observe a significant increment in amino acid efflux across the forearm. Forearm blood flow increased, an event which had also been demonstrated previously by Pozefsky (1976).

Muscle substrate utilization continues to change after an overnight fast. Owen and Reichard (1971) in studies performed across the forearm muscle in 8 obese subjects, demonstrated a change in the uptake of glucose, FFA and ketone bodies. After a 3 day fast, ketone body oxidation accounts for at least 51% of the oxygen extracted by muscle as compared to 10% after an overnight fast. Free fatty acid extraction plays a secondary role to ketones in muscle metabolism after a brief fast. Glucose extraction drops considerably.

Garber (1974) studied splanchnic metabolism in 5 patients after 3 days of starvation. He demonstrated a complimentary substrate interaction between the splanchnic bed and the periphery by documenting an increment in gluconeogenesis. Eighty one percent of the glucose produced by the splanchnic bed was accounted for by the extraction of lactate, pyruvate, glycerol and alanine. Felig (1969) demonstrated that splanchnic extraction of glucogenic amino acids increased after 3 days of fasting. Alanine's extraction fraction increased from 40% after an overnight fast to 70% after 3 days of food deprivation. There is also a significant uptake of serine, glycine, tyrosine, phenylalanine, lysine, and tryptophan. Felig's and Garber's studies assessed the splanchnic bed metabolism but did not specifically study the contribution of the non-hepatic splanchnic bed.

Splanchnic ketogenesis correlates significantly with gluconeogenesis (Garber, 1974). Ketone production by the splanchnic bed is about 1.118 moles/day (Reichard, 1974). Hepatic ketogenesis peaks after 3 days of starvation and continues at that same rate throughout the fast (Felig, 1969). Free fatty acids provide the substrates and energy for ketogenesis. Fifty five percent of the extracted FFA is consumed to form this amount of ketone bodies. Accompanying ketogenesis there is an

increased hepatic uptake of FFA which is due to increased arterial concentrations since their extraction fraction does not change with starvation (Garber, 1974, Fine, 1960). Concurrent with the increased rate of ketogenesis, Owen (1971) found increased ketone utilization by muscle which peaks after 3 days of fasting. Ketone utilization by muscle falls to postabsorptive values after a prolonged fast. The importance of ketone metabolism in man becomes apparent in prolonged starvation. Ketones become the major fuel for the brain and a major stimulus for renal gluconeogenesis (Owen, 1967, Cahill, 1975).

Along with the falling glucose and insulin levels leading to increased gluconeogenesis, lipolysis and ketogenesis, the blood levels of certain amino acids show a particular pattern (Felig, 1969). Except for the branched chain amino acids, the levels of most amino acids begin to drop after a brief fast. The initial elevation of the branched chain amino acids is thought to be due to their decreased metabolic clearance rate accompanied by an unchanged plasma delivery rate (Sherwin, 1978). This peculiarity in BCAA levels is probably mediated by insulin since branched chain amino acidemia has been correlated with insulinopenic states such as uncontrolled diabetes mellitus (Felig, 1970).

The dog, like man, continues to utilize glucose after a brief fast. The estimated splanchnic glucose production is 13 μmoles/kg of body weight/min. or 2.5 mg/kg/min (Keller, 1977, 1978). This value is only 7% less than those obtained by Cherrington in the same laboratory in postabsorptive dogs. Cowan (1969), using radioactive tracers, estimated glucose production in the 3 day fasted dog. The dog produces glucose at a rate of 3 grams/kg/day or 2 mg/kg/min, a value 20% less than those found by Keller (1978). The values for glucose production and therefore utilization are then very similar to those obtained in man.

Canine liver glycogen is depleted after the initial 24 hours of food deprivation (Swenson, 1946). Glycerol and most amino acids except for leucine and the alanine involved in the alanine-glucose cycle are responsible for the net glucose production. Lactate, pyruvate and most of the alanine released from skeletal muscle are part of the Cori and alanine-glucose cycle and do not lead to net glucose production (see above). The balance of amino acids and metabolites across the different tissues of the splanchnic bed have been studied in the briefly fasted dog. This is not the case for man. Keller (1977) observed an uptake of glucose (3.78±.5 μmoles/kg/min), free fatty acids (.53±.22 μmoles/kg/min) and ketone bodies (.26±.04 μmoles/kg/min) across the non-hepatic splanchnic bed. Glycerol was not significantly extracted during a brief fast by the non-hepatic splanchnic bed as compared to a 20% extraction in Basso's (1970) postabsorptive dogs.

Vaidyanath (1978) in 4-7 day fasted anesthesized dogs found that glutamine was the only amino acid extracted by the non-hepatic splanchnic bed. This contrasts with the findings of Weber (1977) who found not only an uptake of glutamine but also serine, leucine, valine, arginine, isoleucine, phenylalanine, tyrosine and histidine in postabsorptive dogs. Vaidyanath also found that β-hydroxybutyrate and pyruvate were removed while glutamate, lactate, alanine, glycine and ammonia were released. He failed to observe the release of proline and citrulline observed by Weber in the postabsorptive dog jejunum.

In order to sustain gluconeogenesis the liver extracts about 550 μmoles of amino acids/liter of plasma (Vaidyanath, 1978). This amount would account for 60% of the glucose produced if we assume, using Keller's data, that glucose production is 13 μmoles/kg/min, that all the amino acids go into glucose production and that whole blood amino acid

concentrations equal those of plasma. Alanine accounts for 40% of the amino acid extracted and could be responsible for 23% of the glucose produced. Histidine, arginine and lysine account for 20% of the amino acids extracted and are responsible for 11% of gluconeogenesis. Vaidyanath also observed that except for the BCAA, glutamine and α-amino-n-butyrate, most amino acids are removed by the liver while pyruvate, triglycerides and carnosine were released. These findings are similar to those reported by Elwyn (1968) in his postabsorptive dogs. Unfortunately, Elwyn's style of data presentation makes it very difficult to make more concrete comparisons of amino acid balances across the liver and extra hepatic splanchnic bed in the postabsorptive and briefly fasted states.

Glycerol plays an important role in gluconeogenesis. Hall (1976) using radioactive tracers found that 13% of the new glucose was produced from glycerol. The relative contribution of glycerol after a brief fast is then 2 times more than after an overnight fast.

Free fatty acids are the fuel of choice for hepatic metabolism. The hepatic uptake of FFA by the splanchnic bed, unlike that of alanine, is concentration dependent. The liver will extract 25% of the FFA presented to it irrespective of the nutritional status of the individual (Fine, 1960, Basso, 1970). Fine (1960) demonstrated that the portal vein FFA concentrations were distinctly higher after 36-40 hours of fasting (959 μEq/liter) than after an 18 hour fast (556 μEq/liter). In the postabsorptive state the liver extracts about 51 μEq of FFA/min (26% extraction fraction) as compared to 80 μEq/min. after a brief fast (27% extraction fraction). The portal FFA concentration reported by Fine are in agreement with the arterial levels (868 μM/l) reported by Keller (1978) in his 48 hours starved animals.

Along with the rising FFA concentration the arterial levels of ketones increase. Arterial ketone levels are three times higher after a 3 day fast than after an overnight fast (31 vs 97 μmoles/l of blood) Basso, 1970, Keller, 1978). The ketone production rate per kilogram of body weight is 10 times higher than during the postabsorptive state (11.7 vs 1.03 μM/kg/min). Along with increasing ketone levels, Keller observed an uptake of β-hydroxybutyrate and acetoacetate across the portal bed in 2 and 3 day fasted animals. Seventeen and thirteen percent, respectively, of the net hepatic production of AcAc and BOHB is attributed to this non-hepatic splanchnic bed uptake (Keller, 1978). The pattern of disappearance for ketone bodies in dogs is very similar to man during a brief fast. At low concentrations the disappearance rates of AcAc and BOHB are proportional to their concentration. But as in man, ketone utilization will fall behind production if their levels are markedly elevated, as in ketotic diabetes mellitus. This is what is seen in man after a prolonged fast (Owen, 1967, Reichard, 1974, Owen, 1971, 1973).

Ketone levels in postabsorptive man and dog are similar (29 μmoles/liter in man vs 31 μmoles/liter in dog) but are very different after a 3 day fast (Cahill, 1966, Basso, 1970, Crandall, 1941). In man 3 days of starvation will be accompanied by ketone concentrations of 2.15 mmoles/liter of blood as compared to the 97 μmoles/liter in the dog (Cahill, 1966, Keller, 1978, Lemieux, 1968). Since the rate of production per kg of body weight is similar, Lemieux(1968) has theorized that the dog metabolizes ketones more efficiently than man.

Glucose balances across the 72 hour fasted anesthesized dogs have shown that the kidney will not produce glucose (Churchill, 1970). Unlike the human kidney, the canine kidney will excrete less urinary ammonia after a brief fast than after an overnight fast (Chruchill, 1970). Unfor-

tunately Churchill and Malvin did not measure urinary nitrogen excretions. Lemieux(1968) studied urinary nitrogen excretion throughout a 12 day fast in dogs and found a fall of both total urinary nitrogen and ammonia excretion accompanied by a rise in urinary pH. This data contrasts markedly with those observed in human subjects who develop ketonemia, and increased urinary ammonia excretion with fasting (Cahill, 1966).

Objectives

Studies on dog interorgan metabolism, unlike those in man, have not included the direct assessment of skeletal muscle in the overall adaptation to food deprivation. The kidney, another important organ in amino acid and glucose homeostasis, has not been fully investigated as it transforms from a glucose consuming organ to a glucose producer. Amino acid balances across this organ have not been measured after a brief fast. And finally, the simultaneous interaction between the different components of the splanchnic bed, skeletal muscle and kidney have not been assessed in the postabsorptive and briefly fasted states. Therefore, the purpose of this study is to measure these complex interorgan fuel-hormone-interactions in those states. In order to do this we have obtained simultaneous arterial and venous samples across the liver, non-hepatic splanchnic bed, kidney and hindleg of conscious dogs with multiple pre-implanted catheters.

Many of the studies in dogs in the past have studied interorgan metabolism during surgery under the effects of anesthesia. Since the effects of anesthesia and surgery on interorgan metabolism are unknown this project was performed in awake, unanesthesized dogs that had recovered from surgery. Moreover, many of the above mentioned studies have measured the metabolite and amino acid concentrations in plasma rather than in whole blood. Studies by Elwyn (1968), Felig (1973) and Aoki (1973) have questioned the importance of the erythrocytes in the transport of amino acids and substrates from one organ to the other. In order to circumvent this possible source of error all the determinations (except for FFA) were performed on whole blood.

Methods

Subjects

Seven trained non-pregnant adult female dogs weighing 18-28 kg were used. Females were selected because of the ease of urinary bladder catheterization. The animals were not selected for breed. They were housed in individual plastic pens at the Harvard Medical School West Quadrangle Animal Facility under vetinary supervision. Lights in the temperature-controlled rooms were on from 0700 to 1900 hours and off from 1900 to 0700 hours. Dogs were fed 666 grams of canned food (Alpo) containing 750 Calories divided into 42% protein, 61% fat and 7% carbohydrate at 1600 hours every day prior to the beginning of the fasting periods. They had free access to water at all times. Dogs wore nylon coats(Chatham Medical Arts) and plastic collars post-operatively to prevent worrying catheter sites. The catheters were flushed daily with 2-3 ml of 250μ/ml of heparin-saline solution. The dogs were walked for 20-30 minutes every day. Rectal temperatures and weight were recorded 2-3 times a week. Special attention was placed on daily food consumption, excretory functions, healing of wounds and the general condition of the animal. Arterial hematocrits were obtained once a week.

Blood cultures, drawn from the catheters and a peripheral vein, were obtained at random or when dogs had temperatures of 39.4°C or higher. Every dog received 5 grains of aspirin and 325 mg of ferrous sulfate daily after taking the rectal temperatures. All dogs had subcutaneous penicillin G injected prophyllactically on the morning of surgery and after each experimental session. No aspirin or iron supplements were given during the fasting periods.

Only 2 of the 7 dogs completed the full experimental protocol. These 2 dogs were studied twice. The other 5 dogs were eliminated because of infection (+ blood cultures, temperatures > 39°C and hematocrit under 30%) or because of malfunctioning catheters.

Surgical Procedure

Operations were performed at the Animal Surgical Suite of the Harvard West Quadrangle Animal Facility. Renal vein, hepatic vein, portal vein, deep femoral vein, infusion and arterial catheters were installed in a single surgical procedure.

On the morning of surgery, the 18 hour fasted animals were sedated with Acepromazoine IM and anesthesized with Nembutal 20 mg/kg of body weight I.V. Following induction of anesthesia a superficial foreleg vein was catheterized with a 20 gauge Jelco unit and a saline infusion was started. The dog received about one liter of fluid during the surgical procedure. One million units of subcutaneous Penicillin G was administered prior to induction of anesthesia.

Under sterile technique a midsagittal abdominal incision was performed. Hepatic and portal catheters were installed by the methods of Shoemaker (1959) as modified by Liljenquist (Keller, 1978). A purse string suture was inserted in the left posterior lobe hepatic vein. The hepatic catheter was inserted through a stab wound and secured with a purse string suture. The portal catheter was threaded through a radicle of the splenic vein into the portal vein up to the point where the latter enters the liver. The left ovarian vein was ligated, the renal vein catheter was inserted into the left renal vein through a stab wound in the vena cava, and secured with a purse string as described by Reed (1971). The deep femoral vein was catheterized by threading a J shaped catheter through the right deep circumflex vein. An infusion

catheter was either installed at the opposite deep circumflex vein or at the internal jugular vein. The arterial catheter was inserted into the aorta through an internal iliac artery or through the common carotid artery.

Catheters were exteriorized in the flank by directing them through long hubless #8 gauge needles, thus creating a subcutaneous tunnel. Injection ports (Jelco) were attached to the ends of the catheters for easy flushing. All catheters were #12 gauge polyethylene with siliastic tips similar to those described by Bergman (Kaufman, 1971).

The dogs were allowed to recover from the operative procedure for at least one week. During this time they were walked and fed daily. They usually recovered quickly and started eating normally by the second post-operative day. During the recovery week the dogs were taught to lie or stand in a modified Pavlov stand.

Experimental Protocol

The animals were fasted for 3 days. Each dog was fasted twice with more than 2 weeks of normal feeding in between fasts. Blood flow, amino acids and metabolite concentration differences across the hindleg, kidney, non-hepatic splanchnic bed and liver were obtained after a 16-18 hour fast and 3 days later. Arterial insulin and glucagon concentrations were also determined. Concentration differences were obtained after the second day but these are not reported here.

The dogs were weighed, walked and fed at their usual feeding time (0016 hours) prior to the first experimental session. After the completion of the meal the food plate was removed. Aspirin and iron were not given during the fast. Water was provided ad.lib. After an overnight fast the dog was taken to the Joslin Research Laboratories. Rectal temperature was obtained after the dog was placed on a modified

Pavlov stand which permitted the dog to lie, sit or stand freely. The urinary bladder was then catheterized and washed with sterile water through a #10 Foley catheter after application of lidocaine to the vagina. The residual heparin in the vascular catheters was withdrawn by suction. A special manifold was attached by means of which normal saline was infused into all the catheters. Total infusion was 500-1,000 cc over a 4-6 hour period. No sedation nor heparinization was necessary.

Blood Flow Determinations

Renal, hepatic and hindleg blood flow determinations were obtained throughout the experimental session. Renal blood flow was estimated by clearances of para-aminohippurate (Harvey, 1962). Hepatic blood flow was calculated using Indocyanine Green (Ketterer, 1960). Hindleg blood flow was determined with the capacitance plethysmograph (See appendix 1). Arterial, hepatic and renal vein bloods were obtained simultaneously.

To calculate the effective renal blood flow, a 3 mg bolus of PAH was injected into the infusion catheter followed by a constant infusion of 2-3 mg/min in order to maintain arterial concentrations between 1-2 mg per deciliter of plasma. After 45 minutes for equilibration, 3 urine specimens were collected after urinary volumes were measured at successive 30 minute intervals. The urinary bladder was washed with sterile water after each urine collection. Blood samples from the renal vein and artery were drawn midway between bladder washings. Blood, urine and plasma were analyzed for PAH by the methods of Harvey (1962). An arterial blood hematocrit in duplicate was obtained in order to calculate the effective renal blood flow by the formula: $RBF = C_{PAH}/1\text{-Hct}$.

Hepatic blood flow was estimated in all experimental sessions except during the third day of the second fast of one of the dogs using the Indocyanine green continuous infusion method (Ketterer, 1960). A bolus of 1.5 mg of

Indocyanine green was injected into the infusion catheter followed by a constant infusion of .58 mg to 1.18 mg/min. After 45 minutes for equilibration, hepatic vein and portal vein samples were collected at successive 30 minute intervals concominantly with the samples for renal blood flow.

Hindlimb blood flow was determined using the capacitance plethysmograph two to three times during the experimental session (Figar, 1959, Aoki appendix 1). Blood flow was not obtained during the postabsorptive period of one of the dogs during the first fast. The screen of the plethysmograph was placed 2 cm above the patella. A pediatric blood pressure cuff was placed one cm above the screen and inflated to 120 mm Hg in order to occlude venous return. The values obtained are reported per 100 cc of hindleg. The relative composition of the dog hindleg was obtained by dissection (see appendix 2).

Blood and Urine Collections and Analysis

Urine samples and volumes were collected 45 minutes after the initiation of PAH infusion and 30 minutes thereafter. The urines were collected four times and analyzed for total urinary nitrogen by the method of Kjeldahl (Wong, 1923) and ammonia by the method of Conway (1939). The first urine collection before initiation of infusion was qualitatively analyzed for ketones, sugar and albumin with a Chemstrip 8 (Bio-Dynamics, Indianapolis, USA).

There were two blood collection periods. The first, 120 minutes after the initiation of the PAH and Indocyanine green infusion and the second 30 minutes later. Eight ml of blood were simultaneously drawn slowly for the artery, portal, hepatic, renal and deep femoral veins and processed for blood ammonia, lactate, pyruvate, citrate, β-hydroxybutyrate, acetoacetate, α-ketoglutarate, glycerol, glutamine and glutamate.

Glucose was analyzed by the hexokinase method from whole blood perchloric acid filtrates (Bergmeyer, 1974). Bloods were processed as described in appendix 3 and stored at -20°C for later analysis. Free fatty acids were analyzed from plasma by the method of Novak (1965). Lactate, pyruvate, citrate, α-ketoglutarate, glycerol, ammonia, β-hydroxybutyrate and acetoacetate were assayed from whole blood by the methods of Bergmeyer (1974). Glutamine and glutamate were analyzed enzymatically and read fluorimetrically (American Instruments, Model 4-7103) by the method of Aoki (1973).

The second blood collection was 30 minutes later. Analyses were done for all metabolites, glucose and free fatty acids plus whole blood amino acids, serum insulin, and plasma glucagon. Serum insulin concentrations were determined in duplicate on 2 ml of arterial blood by the radioimmunoassay double antibody technique (Soeldner, 1965). Plasma glucagon was determined on 2 ml of arterial blood, collected in a tube containing EDTA and trasylol with Unger's 30k antibody (Unger, 1972).

Samples for amino acid were processed from all vessels as described in appendix 3. Concentration determinations (except for glutamine and glutamate) were performed by column chromatography on a Beckman 119 Amino Acid Analyzer modified to sequentially inject samples on two independent Durrum DC-6A resin and columns (bed height 27.5 cm) with Durrum Pico Buffer System IV (lithium) and two temperatures (36.5 and 60°C). A total of 160 cc of blood were drawn per experimental session. This was replaced with normal saline.

Calculations

1) Rates of extraction and/or production were derived by combining regional blood flow with arteriovenous differences and are expressed as μmoles/min per dog for portal, hepatic, net-splanchnic bed and renal bed and as μmoles/100 cc of hindleg per minute across the hindleg.

2) Arteriovenous differences across the liver were calculated by the following formula: (.2A + .8PV) - HV. Hepatic fluxes were determined by the following formula: HBF [(.2A + .8PV) - HV]

3) Statistics were performed by the paired student test, and t values matched to probability values with a double tail normal curve except where noted.

Results

Table 1 shows the date of surgery, pre-operative weight, date of experiment, and weight, rectal temperature and blood hematocrit changes during the fasting periods. Only 2 out of 7 dogs who were operated on completed the experimental protocol. These two dogs, who were in good health, were fasted twice.

The dogs' weights were stable prior to the fasts. They lost 1.7 kg or 8% of their body weight during each fast. They consumed their usual diet in between fasts and would quickly regain their original weight.

Table 1: Animal Data

Dog	Day of Surgery	Pre-op weight	Day of experiment	Experiment week wt*	temp	Hct
White A	7/9/79	20.8				
vernight fast			7/24	20.8	38.3	33%
rd day fast			7/27	19.1	38.6	31%
White B	7/9/79	20.8				
vernight fast			8/14	20.6	38.4	40%
3rd day fast			8/17	18.9	38.5	34%
Chow A	8/1/79	23.2 kg				
vernight fast			8/7	23.	39.4	33%
3rd day fast			8/10	21.3	39.2	27%
Chow B	8/1/79	23.2 kg				
overnight fast			8/21	23.5	38.9	30%
3rd day fast			8/24	21.8	38.4	31%

* Mean weignt lost = 1.7 ± 0 kg ($P \leq .01$) or 8% of body weight

Arterial Blood Concentrations of Substrates and Amino Acids

In table II are summarized the mean values of all constituents measured in whole blood except for insulin and glucagon. Free fatty acids were measured in plasma. Levels of glucose, pyruvate, lactate/pyruvate ratio, α-ketoglutarate, citrate, glycerol and ammonia did not change significantly. There was a significant (34%) fall in lactate concentration. β-Hydroxybutyrate levels increased by 90% but this was not statistically significant. Acetoacetate increased significantly by 78% while the ratio of the two did not change appreciably. Total arterial blood ketone bodies increased by 86% and this was significant ($p<.05$, single tail). Free fatty acids were 36% higher after a 3 day fast and this change was significant if percent change rather than the raw values were analyzed.

Total amino acid levels fell by 14%. Only serine (41%), glycine (30%), valine (40%), leucine (24%), tyrosine (41%), and histidine (24%) fell after a 3 day fast. Alanine concentration decreased by 30% but this difference was not significant. Glutamine was 24% higher after 3 days of starvation but this rise was not significant.

Insulin and Glucagon

Table IIb shows the changes in arterial insulin and glucagon concentrations. There was a significant fall in insulin and glucagon concentration between the postabsorptive and 3 day fast. The insulin and glucagon concentrations did not change significantly between postabsorptive state and the second day of the fast. There was no significant change in the molar ratio of insulin to glucagon.

Table II

Arterial Substrate Concentrations in the Postabsorptive State and After a Brief Fast

	Overnight fast	3rd day fast	P
Glucose mM/L	4.85 ± .1955	4.80 ± .2593	N.S.
Lactate uM/L	401.6 ± 39.5	264.8 ± 6.2	<.05
Pyruvate uM/L	32.6 ± 3.2	29.3 ± 4.6	N.S.
Lactate/Pyruvate Ratio	12.7 ± 1.9	9.6 ± 1.2	N.S.
α-Ketoglutarate uM/L	8.1 ± 3.0	6.5 ± 1.7	N.S.
Citrate uM/L	72.8 ± 33.1	117.9 ± 15.3	N.S.
β-Hydroxybutyrate uM/L	79.8 ± 25.6	151.8 ± 28.9	N.S.
Acetoacetate uM/L	41.8 ± 8.9	74.4 ± 7.2	<.05
β-OH/AcAc Ratio	1.8 ± .224	2 ± .2	N.S.
Glycerol uM/L	109 ± 19.4	93.4 ± 20.2	N.S.
Ammonia (blood) uM/L	97.13 ± 13.96	103 ± 22	N.S.
Taurine uM/L	204.3 ± 25.1	181 ± 16.4	N.S.
Aspartic Acid "	48 ± .8	41.5 ± 4	N.S.
Threonine "	323.8 ± 49.9	276.5 ± 36.7	N.S.
Serine "	157.8 ± 9.5	92.5 ± 5.1	<.02
Asparagine "	35.5 ± 4.7	27.5 ± 4.2	N.S.
Proline "	175.5 ± 35.7	136 ± 5.6	N.S.
Glycine "	201.3 ± 15.3	140.3 ± 13.3	.01
Alanine "	223.3 ± 32.9	169.8 ± 11.6	N.S.
Free Fatty Acids μEq/l	563.3 ± 130.8	792 ± 139.4	N.S.
α-amino-n-butyrate "	41.8 ± 13.2	53.5 ± 12.9	N.S.
Valine "	259 ± 13.7	154.8 ± 8	.005
Cystine "	34.3 ± 8.4	28.5 ± 8.4	N.S.
Total Ketone Bodies "	121.5 ± 34.2	226.1 ± 35.8	.1
Isoleucine "	85.8 ± 4.8	95 ± 15.8	N.S.
Leucine "	157.3 ± 6.5	119.5 ± 5.7	.005
Tyrosine "	53.3 ± 2.9	31.25 ± 4.5	.05
Phenylalanine "	49 ± 7.9	37 ± 4.6	N.S.
Ornithine "	32.8 ± 3.9	27.8 ± 7.7	N.S.
Lysine "	211.5 ± 13.2	183 ± 14.5	N.S.
Histidine "	73 ± 4.8	55.5 ± 4.9	.05
3-Methyl histidine "	16 ± 5.7	21 ± 5.1	N.S.
Arginine "	136.7 ± 13	105.2 ± 21.2	N.S.
Glutamine (enz) ! "	424.8 ± 56.9	527.8 ± 38.2	N.S.
Glutamic acid (enz) "	70.1 ± 5.8	77.9 ± 5.1	N.S.
Phosphoserine "	53 ± 7.9	66.8 ± 9.9	N.S.

*P = probability that A_{PA} does not differ from $A_{3rd\ day\ fast}$ (paired t-test)

‡ mean ± SEM uM/liter of whole blood

* μEq/liter of plasma

! Glutamine concentrations, n=3

Table IIb

Difference in Insulin, Glucagon and Insulin/Glucagon Ratio

Between the Postabsorptive State and a 3 Day Fast

Insulin μU/ml	WA	WB	CA	CB	Mean±SEM	
Overnight Fast	14	13	22	19	17±2.12	
3rd Day Fast	8	11	11	11	10.25±.75	p=.05
Glucagon pg/ml						
Overnight Fast	50	80	60	70	65±6.45	
3rd Day Fast	20	40	20	50	32.5±7.5	p=.01
Insulin/Glucagon						
Overnight Fast	6.53	3.79	8.56	6.33	6.30±.98	
3rd Day Fast	9.33	6.42	12.833	5.13	8.43±1.71	

Splanchnic and Portal Bed Arteriovenous Differences in the Postabsorptive State

Table III shows the splanchnic arteriovenous differences for substrates and amino acids after an overnight fast (16-18 hours). Free fatty acids are not reported since only arterial levels were assayed. The splanchnic bed (A-HV) tends to produce glucose (105±66.4 μM/liter) but this difference is not significant. A significant release of leucine and valine from this bed was noted. There was a tendency of this bed to consume α-ketoglutarate and to produce acetoacetate. The BOHB/AcAc ratio decreased as blood crossed the splanchnic bed (.16±.04).

Table III also reveals the exchange of substrates and amino acids across the portal vein drained viscera. Pyruvate, ammonia, alanine and glutamate were significantly released. Lactate showed a tendency to be released ($p=.1$). β-Hydroxybutyrate was extracted ($p<.05$). Glutamine extraction averaged 24.3±23.2 μm/liter but this difference was not significant. Glucose consumption averaged 178.6±141.2 μmoles/liter of blood but this was not statistically significant. Isoleucine, leucine and threonine showed a tendency to be produced ($p<.1$) while all the other amino acids and metabolites were not significantly extracted or released.

Hepatic Balances [(.2A + .8PV) - HV] and Extraction Fractions in the Postabsorptive State and After a Brief Fast

Table IV shows the arterio hepatic venous differences and extraction fractions for all substrates and amino acids. All were assayed in whole blood. Free fatty acids, methionine and citrulline are not reported. After an overnight fast the liver released significant amounts of glucose while it removed pyruvate and taurine. The pyruvate extraction fraction was 46%, and 15% of the taurine influx was extracted. Alanine, lactate and glycerol were not significantly produced or extracted. Ketone body

Table III

Splanchnic and Portal Bed Arteriovenous Differences

in the Postabsorptive State

	A-HV	P	A-PV	P
Glucose uM/L	-105. ± 66.40	NS	178.6 ± 141.2	NS
Lactate uM/L	61.5 ± 36.1	NS	-75.6 ± 28	.1
Pyruvate uM/L	7.5 ± 3	NS	-17.5 ± 4.7	<.05
Lactate/Pyruvate Ratio	-1.44 ± 1.1	NS	2.99 ± 99	.05
α-Ketoglutarate uM/L	2.4 ± .8	.1	-1.4 ± 1.8	NS
Citrate uM/L	-8.3 ± 6.7	NS	4.4 ± 4.3	NS
β-Hydroxybutyrate uM/L	-38.8 ± 19.3	NS	9.5 ± 2.9	<.05
Acetoacetate uM/L	-25.8 ± 10.5	.1	9.9 ± 4.4	NS
β-OH/AcAc Ratio	.16 ± .04	<.05	-.28 ± .19	NS
Glycerol uM/L	32.8 ± 15.8	NS	5.9 ± 6.5	NS
Ammonia (blood) uM/L	-.12 ± 7.6	NS	-92 ± 9.5	<.01
Taurine uM/L	33.8 ± 23.3	NS	7.5 ± 30.3	NS
Aspartic Acid "	-.5 ± 1.9	NS	4.3 ± 3.1	NS
Threonine "	-16.5 ± 5.8	NS	-30.8 ± 11.3	.1
Serine "	-4.3 ± 12.2	NS	-15.8 ± 15.1	NS
Asparagine "	-6.3 ± 3.7	NS	-11.5 ± 6.1	NS
Proline "	20.5 ± 14.1	NS	-8.3 ± 25.2	NS
Glycine "	-3 ± 6.8	NS	-42 ± 20.3	NS
Alanine "	-2.5 ± 10.2	NS	-84.3 ± 26.2	<.05
Citrulline "	-1.33 ± 1.2	NS	-3 ± 14.7	NS
α-amino-n-butyrate "	-1.5 ± 1.6	NS	-4 ± 2.1	NS
Valine "	-19.8 ± 3.2	<.01	-23 ± 15	NS
Cystine "	1.25 ± 1.4	NS	-1.5 ± 2.3	NS
Methionine "	-2 ± .6	.1	-.33 ± 4.3	NS
Isoleucine "	-6 ± 3.5	NS	-10 ± 4.2	.1
Leucine "	-18.3 ± 5.4	<.05	-29 ± 11.9	.1
Tyrosine "	.8 ± 1.5	NS	5.30 ± 4.7	NS
Phenylalanine "	0 ± 3.7	NS	-15.5 ± 7.5	NS
Ornithine "	2.5 ± 1.5	NS	3.5 ± 2.9	NS
Lysine "	-12.75 ± 7.2	NS	-22.3 ± 12.7	NS
Histidine "	2.8 ± 2.9	NS	-4.3 ± 6.1	NS
3-Methyl histidine "	-.5 ± 1.7	NS	-1 ± 1	NS
Arginine "	1.5 ± 4.3	NS	-12.3 ± 8.7	NS
Glutamine (enz) "	-7.2 ± 8.2	NS	24.3 ±23.2	NS
Glutamic acid (enz) "	-.4 ± 6.5	NS	-10.3 ± 1.4	<.01
Phosphoserine "	- 4 ± 5.6	NS	-11.8 ± 6.1	NS

* Mean ± SEM, μM/liter of whole blood

P = paired t test n=4 except for GlN; n=3.

balances across the liver were not significant. The ratio of lactate to pyruvate increased as the blood crossed the liver (-3.825±.997). The branched chain amino acids as well as glutamine and glutamate were neither released nor produced.

Three days later the arterial hepatic venous difference for glucose was not significantly different from zero but the mean A-HV difference was similar to that after an overnight fast. Pyruvate extraction continued to be significant but the ratio of lactate/pyruvate did not change significantly. The extraction fraction for pyruvate did not change appreciably. The extraction fraction for aspartate was significantly different suggesting increased avidity of the liver for this amino acid. The extraction fraction for alanine was 46%, the highest for any amino acid but was not significantly different from the alanine extraction fraction of the postabsorptive period.

Splanchnic and Portal Bed Arteriovenous Differences After a Brief Fast

Table V shows the splanchnic and portal bed substrate and amino acid differences after a 3 day fast. The splanchnic bed tends to produce glucose (170 μmoles/liter) but as in the postabsorptive period this difference was not significant. It extracts significant amounts of proline but no other amino acid. Valine and isoleucine tended to be produced ($p<.1$). There was no change in the ratio or BOHB/AcAc across this 3 day fasted bed as there was in the postabsorptive period.

The portal drained viscera extracted β-hydroxybutyrate and proline, and released pyruvate and glutamate. Alanine tended to be released while glutamine tended to be extracted.

Table IV

Hepatic Balances [(.2A + 8PV) - HV] and Extraction Fraction $\frac{(.2A+8PV)-HV}{(.2A+8PV)}$

in the Postabsorptive State and After a 3-Day Fast

	Postabsorptive State		
	(2A + .8P) - HV		Extraction Fraction
Glucose uM/L	-.283 ± -.08	.05	-6.418 ± 2.083
Lactate uM/L	122 ± 47.46	NS	25.762 ± 10.821
Pyruvate uM/L	21.5 ± 6.28	.05	45.491 ± 11.305
Lactate/Pyruvate Ratio	-3.825 ± .997	.05	-38.692 ± 12.083
α-Ketoglutarate uM/L	3.48 ± 1.33	NS	45.290 ± 18.878
Citrate uM/L	-25.579 ± 20.493	NS	-1.33 ± 6.322
β-Hydroxybutyrate uM/L	-46.35 ± 21.103	NS	-55.749 ± 15.279
Acetoacetate uM/L	-33.65 ± 13.479	NS	-88.414 ± 22.353
β-OH/AcAc Ratio	.387 ± .195	NS	16.796 ± 5.741
Glycerol uM/L	28.05 ± 15.977	NS	24.109 ± 13.399
Ammonia (blood) uM/L	73.475 ± 11.629	.01	43.05 ± 6.365
Taurine uM/L	27.75 ± 8.291	NS	14.457 ± 4.934
Aspartic Acid "	-3.9 ± 3.353	NS	-9.536 ± 7.39
Threonine "	8.100 ± 11.583	NS	2.720 ± 3.053
Serine "	8.350 ± 4.143	NS	5.113 ± 2.698
Asparagine "	2.950 ± 1.769	NS	6.333 ± 4.383
Proline "	29.350 ± 6.582	.05	16.028 ± 4.334
Glycine "	30.6 ± 14.654	NS	11.722 ± 5.220
Alanine "	64.9 ± 18.485	.05	21.081 ± 3.421
Citrulline "			
α-amino-n-butyrate "	1.700 ± 2.213	NS	7.090 ± 5.710
Valine "	-1.350 ± 14.873	NS	-.449 ± 5.406
Cystine "	2.450 ± 3.183	NS	1.904 ± 8.136
Methionine "			
Isoleucine "	2.000 ± 6.569	NS	2.317 ± 6.714
Leucine "	4.950 ± 13.300	NS	2.390 ± 7.301
Tyrosine "	6.350 ± 3.301	NS	10.047 ± 5.072
Phenylalanine "	12.400 ± 3.712	.05	20.584 ± 7.508
Ornithine "	-.3 ± 3.044	NS	-6.166 ± 12.542
Lysine "	5.050 ± 13.162	NS	1.411 ± 5.601
Histidine "	6.150 ± 4.371	NS	7.584 ± 6.012
3-Methyl histidine "			
Arginine "	11.300 ± 4.470	.1	7.964 ± 2.951
Glutamine (enz) * "	-26.63 ± 13.4	NS	-6.52 ± 3.034
Glutamic acid (enz) "	7.825 ± 5.993	NS	8.732 ± 6.676
Phosphoserine "	5.4 ± 4.437	NS	6.857 ± 8.921

P = paired t-test, n=4

values = $\bar{x}$ ± SEM, μMoles/liter of blood

*glutamine determinations in 3 dogs only

P‡ paired t-test, P.A. vs 3rd day

A = arterial concentration

PV = portal concentration

HV = hepatic vein concentration

Table IVb

3-Day Fast

	(.2A + .8PV) - HV		Extraction Fraction	PI
Glucose uM/L	-212 ± -118	NS	-5.007 ± 3.096	NS
Lactate uM/L	64.025 ± 26.873	NS	20.835 ± 9.153	NS
Pyruvate uM/L	12.075 ± 2.395	.02	35.762 ± 10.055	NS
Lactate/Pyruvate Ratio	-2.438 ± .907	.1		
α-Ketoglutarate uM/L	1.513 ± 2.323	NS	25.353 ± 28.153	NS
Citrate uM/L	-2.925 ± 3.727	NS	-1.617 ± 3.271	NS
β-Hydroxybutyrate uM/L	-89.475 ± 43.350	NS	-61.516 ± 30.045	NS
Acetoacetate uM/L	-38.608 ± 25.547	NS	-52.790 ± 29.766	NS
β-OH/AcAc Ratio	-.134 ± .086	NS		
Glycerol uM/L	1.97 ± 18.922	NS	18.233 ± 15.099	NS
Ammonia (blood) uM/L	72.225 ± 25.497	.1	46.47 ± 9.2	NS
Taurine uM/L	-13.100 ± 15.531	NS	-7.474 ± 8.073	NS
Aspartic Acid "	1.650 ± 2.427	NS	3.980 ± 4.827	.01
Threonine "	18.750 ± 30.582	NS	2.906 ± 8.715	NS
Serine "	-1.150 ± 10.003	NS	-2.248 ± 9.534	NS
Asparagine "	-4.050 ± 5.952	NS	-20.82 ± 23.097	NS
Proline "	-13.700 ± 7.735	NS	-17.780 ± 11.374	.05
Glycine "	9.500 ± 5.574	NS	6.624 ± 3.783	NS
Alanine "	24.850 ± 16.180	NS	11.517 ± 8.184	NS
Citrulline "				
α-amino-n-butyrate "	3.8 ± 10.615	NS	-13.808 ± 29.276	NS
Valine "	-1.750 ± 12.953	NS	-5.065 ± 9.888	NS
Cystine "	-6.150 ± 8.201	NS	-28.792 ± 43.012	NS
Methionine "				
Isoleucine "	-2.650 ± 2.417	NS	-3.611 ± 3.223	NS
Leucine "	-18.150 ± 10.251	NS	-13.406 ± 6.895	NS
Tyrosine "	1.750 ± 1.740	NS	3.787 ± 4.432	NS
Phenylalanine "	4.55 ± 4.008	NS	10.787 ± 10.629	NS
Ornithine "	1.900 ± 3.121	NS	5.426 ± 16.131	NS
Lysine "	13.450 ± 9.997	NS	8.453 ± 6.859	NS
Histidine "	12.950 ± 8.520	NS	24.480 ± 16.967	NS
3-Methyl histidine "				
Arginine "	5.250 ± 7.403	NS	3.311 ± 7.254	NS
Glutamine (enz) "	-17.43 ± 9.602	NS	3.602 ± 2.1325	NS
Glutamic acid (enz) "	2.675 ± 2.509	NS	3.16 ± 3.15	NS
Phosphoserine "	4.500 ± 4.772	NS	5.490 ± 7.573	NS

Table V

Splanchnic and Portal Bed Arteriovenous Differences

During a Brief Fast (3 Days)

	A-HV	P	A-PV	P
Glucose uM/L	-170 ± 192.06	NS	-87 ± 141.3	NS
Lactate uM/L	25.1 ± 20.5	NS	14.1 ± 20.8	NS
Pyruvate uM/L	3.9 ± 3	NS	-10.3 ± .9	.01
Lactate/Pyruvate Ratio	-1.4 ± 9	NS	1.3 ± .8	NS
α-Ketoglutarate uM/L	-1.1 ± 2.1	NS	-.9 ± .7	NS
Citrate uM/L	-1.1 ± 3.9	NS	2.3 ± 2.6	NS
β-Hydroxybutyrate uM/L	-71.9 ± 46.8	NS	22 ± 6.8	.05
Acetoacetate uM/L	-34.2 ± 27.2	NS	5.5 ± 3.3	NS
β-OH/AcAc Ratio	-.03 ± .02	NS	.13 ± .1	NS
Glycerol uM/L	25.5 ± 19.2	NS	7.25 ± 5.37	NS
Ammonia (blood) uM/L	22.1 ± 14.7	NS	-62.63 ± 18.05	.05
Taurine uM/L	-19.5 ± 17.43	NS	-8 ± 23.11	NS
Aspartic Acid "	3.25 ± 4.6	NS	2 ± 6	NS
Threonine "	2.75 ± 20.58	NS	-20 ± 21	NS
Serine "	-3.75 ± 9.5	NS	-3 ± 14	NS
Asparagine "	7.25 ± 6.02	NS	-4 ± 5	NS
Proline "	33.5 ± 3.2	.01	59 ± 12	.01
Glycine "	.50 ± 3.48	NS	-11 ± 11	NS
Alanine "	.25 ± 4.82	NS	-31 ± 17	NS
Citrulline "			-8 ± 5	NS
α-amino-n-butyrate "	3 ± 3.44	NS	-1 ± 11	NS
Valine "	-7.75 ± 2.50	.1	-7.5 ± 19	NS
Cystine "	-8.75 ± 7.18	NS	-3 ± 8	NS
Methionine "	0 ± 2.08	NS	1 ± 2	NS
Isoleucine "	-7.25 ± 2.65	.1	-6.75 ± 5.2	NS
Leucine "	-26.75 ± 12.57	NS	-11 ± 4	NS
Tyrosine "	-3.25 ± 3.12	NS	-6 ± 2	NS
Phenylalanine "	-1.25 ± 3.77	NS	-7 ± 3	NS
Ornithine "	3.5 ± 5.56	NS	2 ± 4	NS
Lysine "	4.25 ± 17.68	NS	-11.5 ± 15	NS
Histidine "	5.25 ± 11.66	NS	-.25 ± 6	NS
3-Methyl histidine "	4 ± 3.49	NS	.25 ± 5	NS
Arginine "	-3.75 ± 8.4	NS	-11 ± 10	NS
Glutamine (enz) "	-.17 ± 16.6	NS	21.3 ± 10.8	NS
Glutamic acid (enz) "	-2.62 ± 2.87	NS	-2.8 ± .6	.01
Phosphoserine "	5.5 ± 4.84	NS	1.3 ± 7	NS

Mean ± SEM, uM/liter of whole blood
P = Pair t-test, n=4 except for Glutamine; n=3

Renal Substrate and Amino Acid Balances After an Overnight and 3 Day Fast

Table VI shows the arterial renal venous differences and fluxes across the kidney after 16-18 hours and 3 days of food deprivation. After an overnight fast the kidney does not produce or extract glucose, lactate, pyruvate, α-ketoglutarate or glycerol. Citrate was extracted significantly while serine, alanine and glutamate were produced. β-Hydroxybutyrate was consumed but there was no change in the ratio of BOHB to AcAc across the kidney. Ammonia was significantly produced.

Three days later, the kidney did not produce glucose. Citrate and β-hydroxybutyrate continued to be significantly extracted but not significantly different from the values obtained after an overnight fast. Serine and glutamate, but not alanine were released and the rates of production again failed to differ from postabsorptive rates. There was a significant decline in the lactate to pyruvate ratio across the kidney. Lactate showed a strong tendency to be extracted ($p<.1$), while pyruvate was released ($p<.1$). After 3 days of fasting the kidney produced significantly more pyruvate than that produced after an overnight fast. It also consumed less α-amino-n-butyrate. Ammonia fluxes are expressed as the total ammonia produced by the kidney (urinary + (A-RV) RBF). The kidney continued to produce NH_3 significantly after 3 days of starvation but this difference was not significantly different from postabsorptive values. Total excreted urinary nitrogen was 63% less after 3 days. Urinary ammonia excretion was 41% less after 3 days but this difference was not significant.

Arteriovenous Differences Across the Postabsorptive and Briefly Fasted Hindleg

Table VII summarizes the data on the exchange of substrates across this tissue. There was a net uptake of glucose, β-hydroxybutyrate and acetoacetate with a release of α-ketoglutarate. Glutamine, serine, alanine

Table VI

Renal Substrate Balance in the Postabsorptive and Briefly Fasted State

	Postabsorptive State			3rd Day of Fast			
	A-RV	P$^{\psi}$	Flux*$^{\phi}$	A-RV	P	Flux	P‡
Glucose uM/L	-5.7±142.9	NS	-9.3±37.2	-251.3±148.2	NS	-68.2±39.3	
Lactate uM/L	30±35	NS	14.7±12.7	26.8±12.4	.1	7.6±3.5	
Pyruvate uM/L	3±2.94	NS	1.115±1.243	-11.6±3.7	.1	-3.018±.994	.05
Lactate/Pyruvate Ratio	.87±1.92	NS		3.3±.5	.01		
α-Ketoglutarate uM/L	2.9±1.4	NS	.339±.320	1.4±1.1	NS	.343±.320	
Citrate uM/L	19.4±3.1	.01	6.1±2.5	34.9±10.5	.05	9.8±3.1	
β-Hydroxybutyrate uM/L	10.8±3.5	.05	4.2±1.4	39.5±7	.02	3.8±1.0	
Acetoacetate uM/L	8.4±3.9	NS	3.6±1.8	14.5±4.9	.1	4.1±1.4	
β-OH/AcAc Ratio	-.13±18	NS		-.08±.1	NS		
Glycerol uM/L	33.2±17.1	NS	6.1±2.5	19.8±8	.1	5.4±2.4	
Ammonia (blood) uM/L	-47.4±8.6	.02	-42.7±7.9	-16.88±4.13	.05	-20.63±3.81	
Taurine uM/L	23.3±31.4	NS	5.8±12.1	-23±34	NS	-4.4±8.1	
Aspartic Acid "	2.3±4.4	NS	.3±1.6	.5±1.55	NS	.2±.4	
Threonine "	-7±12.3	NS	-3±5.7	-21±11	NS	-2.4±2.1	
Serine "	-50.5±10.3	.02	-20.4±6.3	-42±8	.05	-11.1±2.4	
Asparagine "	-8.3±4.9	NS	-3.4±2.3	-9±7	NS	-2.6±2	
Proline "	24±11.5	NS	6.7±4.6	52±19.4	.1	13.1±4.2	
Glycine "	21.8±8.5	.1	.245±4.918	21±12	NS	5.1±2.7	
Alanine "	-36.8±3.5	.01	-14.39±2.93	-39±18 Ω	NS	-11±5.3	
Citrulline "	3.7±2.3	NS	1.2±.8	3.5±.5	.1	1.0±.1	
α-amino-n-butyrate "	-5±3	NS	1.665±.917	2±3.5	NS	.403±.890	.001
Valine "	-10.8±6	NS	-4.4±2.6	-12.5±7	NS	-3.5±2.2	
Cystine "	-5.3±5.5	NS	-2.1±2.3	-11±7	NS	-3.0±2.0	
Methionine "	4±3.5	NS	1.2±1	.34±1.2	NS	.059±.340	
Isoleucine "	0±3.6	NS	-.423±1.3	-7±5	NS	-2.1±1.5	
Leucine "	-2±2.6	NS	-1.1±1.1	-13±4	.1	-3.6±1.3	
Tyrosine "	-3±2.1	NS	-1.3±.8	-6±3	NS	-1.7±.9	
Phenylalanine "	-6±3.9	NS	-2.8±1.9	-8±3	NS	-2.1±.9	
Ornithine "	-1.5±1.9	NS	-.8±.8	-.75±8	NS	.07±2.3	
Lysine "	-8.3±10	NS	-3.5±4.1	7.25±15	NS	1.9±4.4	
Histidine "	-7.3±2.9	.1	-2.9±1.5	-7±5	NS	-1.9±1.4	
3-Methyl histidine "	-1±.4	.1	-.38±.249	-1.67±3.18	NS	-.46±.888	
Arginine "	-9.3±6	NS	-4.1±2.6	-5.75±10	NS	-1.7±2.9	
Glutamine (enz) "	36.2±32	NS	10±11.0	22.5±7.9	.1	6.4±2.2	
Glutamic acid (enz) "	-9.1±1.1	.01	-3.4±.6	-14.3±1.1	.01	-3.8±.4	
Phosphoserine "	-6.3±3.2	NS	-1.8±1.6	1.25±6.5	NS	.3±1.8	
Urinary NH_3			23.67±7.07			14.02±3.93	NS
Urinary Nitrogen mg/min			8.9±1.2			3.3±.4	.05

* Flux = Mean ± SEM, μM/min

ø Total (urinary + renal vein) NH_3 flux

ψ Paired t-test, renal vein concentration is no different from arterial concentration

‡ Paired t-test, $flux_{PA}$ no different from $flux_{3rd\ day}$

Ω Range (-6 to -92)

and the branched chain amino acids, as well as the other amino acids were not significantly extracted or released by this tissue when arteriovenous differences are analyzed. Glutamine was significantly released when fluxes (n=3) rather than arteriovenous differences were analyzed.

After 3 days of starvation there was a significant release of lactate ($p < .01$), α-ketoglutarate ($p < .02$), valine ($p < .02$) and cystine ($p < .02$). β-Hydroxybutyrate and acetoacetate were extracted by the hindleg ($p < .01$) and tne β-hydroxybutyrate/acetoacetate ratio increased across this tissue indicating that it was more reduced than the rest of the body ($-.24 \pm .04$, $p \leq .01$). There was a tendency for glutamine release (-32.3 ± 10.3, $p \leq .1$) while alanine was neither extracted nor produced ($-.5 \pm 2.5$ μmoles/liter).

Hemodynamic Data

Table VIII shows the blood flow values for the splanchnic bed, kidney and hindleg after an overnight fast and after a 3 day fast. There was no significant change in blood flow per kilogram of body weight in the different tissues after a 3 day fast. The hindleg blood flow tended to increase ($p < .1$) after 3 days of starvation. This tendency has been described in man by Finley (1980), Owen (1971), and Pozefsky (1969). The renal extraction fraction for PAH was 82%.

Splanchnic and Non-Hepatic Splanchnic Bed Substrate and Amino Acid Fluxes During the Postabsorptive State and a Brief Fast

Table IX shows the fluxes of substrates and amino acids across the splanchnic bed during the postabsorptive state and after a brief fast. Net glucose production, even though not statistically significant, increased by 70% after 3 days of fasting. This change was mainly due to decreased glucose uptake by the non-hepatic splanchnic bed. The ratio of BOHB/AcAc tended to increase ($p = .1$) as blood crossed this bed. The BCAA tended to be released with valine flux being significantly lower after 3 days of

Table VII

Hindleg Substrate Balance in the Postabsorptive and Briefly Fasted State

	Overnight Fast			3 Day Fast			
	A-DF	P	Flux	A-DV	P	Flux	P^{Ψ}
Glucose uM/L	192.9±113.8	NS	.838±.834	7.5±120.7	NS	.313±2.669	
Lactate uM/L	-42.9±25.4	NS	-.421±.0258	-65.1±7.8	.01	-.53±.181	
Pyruvate uM/L	-3±1.6	NS	-.018±.018	-4.6±3.2	NS	-.018±.012	
Lactate/Pyruvate Ratio	.31±1.16	NS	-.810±.430	-1.25±.6	NS	-.870±1.173	
α-Ketoglutarate uM/L	-2.6±.7	.02	-.006±.009	-2.6±.6	NS	-.031±.078	
Citrate uM/L	-2.5±3.3	NS	-.001±.035	-2.1±4.3	NS	-.015±.062	
β-Hydroxybutyrate uM/L	16.1±4.9	.05	.109±.015	30.5±4.7	.01	.314±.086	
Acetoacetate uM/L	8±.8	.01	.047±.010	21.5±1.6	.01	.186±.037	.05
β-OH/AcAc Ratio	.03±.08	NS	.083±.079	-.24±.04	.01	-.220±.050	
Glycerol uM/L	-5.9±4.8	NS	-.076±.048	-41±12	NS	-.449±.208	
Ammonia (blood) uM/L	10.13±11.1	NS	.013±.061	21.8±19.9	NS	.416±.218	
Taurine uM/L	-3.25±23.2	NS	-,20±.275	-52±30.7	NS	-.585±.371	
Aspartic Acid "	2.75±4.1	NS	-.011±.038	4.25±3	NS	.029±.041	
Threonine "	-22.5±19.1	NS	-.247±.273	-6.75±9.58	NS	-.006±.085	
Serine "	1.75±12.34	NS	-.061±.145	-3.5±8.87	NS	.008±.088	
Asparagine "	-.5±3.77	NS	-.017±.045	-1.5±2.96	NS	-.032±.030	
Proline "	3±9.33	NS	.066±.047	43.5±9.15	.05	.344±.152	
Glycine "	3.75±13.79	NS	-.052±.129	0±2.74	NS	.014±.026	
Alanine "	-6±15.81	NS	-.098±.154	-.5±2.6	NS	-.022±.015	
Citrulline "	5±3.46	NS	.040±.023	7±3	NS	.041±.005	+
α-amino-n-butyrate "	1.5±2.9	NS	.016±.021	3.75±4.46	NS	.024±.028	
Valine "	9.25±9.38	NS	-.005±.087	-15±3.14	.02	-.532±.391	
Cystine "	3.25±4.7	NS	.003±.029	-10±1.78	.02	-.039±.052	
Methionine "	.67±2.33	NS	-.005±.005	-3.66±4.81	NS	-.028±.047	+
Isoleucine "	5.75±5.39	NS	-.008±.027	-4±2.8	NS	-.007±.030	
Leucine "	4.75±5.02	NS	-.009±.038	-7±5.03	NS	-.081±.059	
Tyrosine "	1.25±3.01	NS	.008±.019	-4.75±2.25	NS	-.035±.028	
Phenylalanine "	-2.25±3.33	NS	-.036±.045	-6.25±2.29	.10	-.041±.027	
Ornithine "	4.75±2.95	NS	.008±.005	7±6.28	NS	.018±.047	
Lysine "	-.25±11.46	NS	-.076±.066	1.25±14.39	NS	-.026±.185	
Histidine "	5.25±4.17	NS	-.001±.036	.25±3.8	NS	-.017±.035	
3-Methyl histidine "	-1±1.08	NS	-.006±.006	3±6.08	NS	.066±.026	+
Arginine "	-6.5±3.86	NS	-.065±.057	-3.75±8.25	NS	.044±.123	
Glutamine (enz) "	-20.8±20.3	NS	-.182±.029	-32.5±10.3	.10	-.298±.118	ø
Glutamic acid (enz) "	-1±.7	NS	-.011±.003	1.9±1.9	NS	.028±.032	
Phosphoserine "	-12.75±7.81	NS	.033±.027	9±3.67	.10	.079±.038	
Total Amino Acids Released			.6975±1.599			.9960±.5296	

* Values = $\bar{X}$ ± SEM, μmoles/100 ml of hindleg/min

\+ Unpaired t-test

Ψ Paired t-test, 3 dogs; Probability that $Flux_{PA}$ does not differ from $Flux_{3rd\ day}$

ø Glutamine flux, pair t-test, n=2

Table VIII

Hemodynamic Values

Overnight Fast	Dog	Liters/min	body weight(kg)	Liters/kg/min
Splanchnic bed	WA	.7996	20.8	.03844
	WB	.8905	20.6	.04323
	CA	1.1506	23	.05003
	CB	.8903	23.5	.03789
Mean±SEM		.93275±.07570		.04240±.00281
Kidney	WA	.3227		.01551
	WB	.4269		.02072
	CA	.28925		.01258
	CB	.4804		.02044
Mean±SEM		.3798 ±.0445		.01731±.00198
Hindleg	WA	5.7		
ml/100cc of hindleg/min	WB	3.4		
	CA	*		
	CB	10.4		
Mean±SEM		6.5±2.06		
3 Day Fast				
Splanchnic bed	WA	.9324	19.1	.04882
	WB	.8353	18.9	.04420
	CA	1.9916	21.3	.09350
	CB	*		
Mean±SEM		1.2531±.57735		.049±.026
Kidney	WA	.2819		.01476
	WB	.2938		.01555
	CA	.2784		.01307
	CB	.2063		.00946
Mean±SEM		.2651±.01988		.01321±.00135
Hindleg	WA	9.05		
	WB	4.6		
ml/100cc of hindleg/min	CA	13.7		
	CB	13.1		
Mean±SEM		10.23±2.09		

*=not measured

starvation. Release of leucine, the only non-gluconeogenic amino acid, as well as isoleucine did not increase after a 3 day fast.

Table IX also shows the non-hepatic splanchnic bed substrates and amino acid fluxes after an overnight and a 3 day fast. The non-hepatic splanchnic bed consumed glucose (even though not significantly) after an overnight fast. Glucose was produced after a 3 day fast but this glucose production was not significantly different from zero. Glucose balances between the postabsorptive state and a brief fast were not statistically significant. Thus decreased glucose consumption by the non-hepatic splanchnic bed after a 3 day fast was accompanied by a 53% increment in β-hydroxybutyrate consumption. Proline, which was not released or produced during the postabsorptive state, was extracted. This difference again was not signfiicant. Alanine efflux from the non-hepatic splanchnic bed did not change after a 3 day fast. Valine, leucine and isoleucine efflux did not change. Glutamine and glutamate fluxes remained relatively stable. α-Amino-n-butyrate showed a strong tendency to be released more after a 3 day fast (p=.1).

Substrate Fluxes Across the Liver in the Postabsorptive and Briefly Fasted State

Table X shows the substrate fluxes across the liver in the postabsorptive and briefly fasted state. Glucose production fell after a 3 day fast but this difference was not signfiicant. Lactate and pyruvate fluxes decreased, though not significantly, after 3 days of starvation. Total extracted gluconeogenic substrates (lactate, pyruvate, citrate, α-ketoglutarate and glycerol) averaged 201±50 μM/min during the postabsorptive state as compared to 142±25 μM/min (p=.1) after a 3 day fast. Total amino acid extraction (excluding leucine) before and after a 3 day

Table IX

Splanchnic and Non-Hepatic Splanchnic Bed Substrates and Amino Acid Fluxes After an Overnight and a Brief Fast

	Splanchnic Bed			Non-Hepatic Splanchnic Bed		
	Overnight Fast	3rd Day Fast	P[Ψ]	Overnight Fast	3rd Day Fast	P
Glucose uM/L	-114.8±74.2	-388.9±160.1	NS	214.9±143.8	-135.6±105.7	NS
Lactate uM/L	87.7±39.9	32.1±23.7	NS	-73.5±14	-63.8±20	NS
Pyruvate uM/L	9.3±.9	4.6±3.8	NS	-15.7±3.4	-10±3.8	NS
Lactate/Pyruvate Ratio			NS			
α-Ketoglutarate uM/L	2.7±.9	2.9±1	NS	-.753±2.043	-.934±.583	NS
Citrate uM/L	-25.4±21.3	-.43±6.25	NS	1.73±4.76	-.56±.66	NS
β-Hydroxybutyrate uM/L	-44.9±14.3	-90.2±50.5	NS	6.64±2.50	14.27±5.3	NS
Acetoacetate uM/L	-29.9±6.3	-39.5±26.5	NS	7.32±3.70	5.04±2.05	NS
β-OH/AcAc Ratio						
Glycerol uM/L	41.8±16.2	27.9±25	NS	3.57±7.23	.43±4.70	NS
Ammonia (blood) uM/L	-.6±8.9	5.8±16.9	NS	-76.9±14.3	-49.8±22.9	NS
Taurine uM/L	52.4±22.4	23.6±33	NS	24.4±17.6	4.2±18.5	NS
Aspartic Acid "	1.2±.7	7.8±5.2	NS	2.4±3	4.6±6.6	NS
Threonine "	.12±11.2	8.2±25.1	NS	20.5±11.7	-22.3±22.6	NS
Serine "	4.2±8.1	-6.4±16.8	NS	-3.7±8.4	-5.9±14.2	NS
Asparagine "	-4.5±4.7	-13.3±14	NS	-7.5±6.8	-10.9±7.4	NS
Proline "	17.3±16.6	42.1±16.2	NS	-13.3±26.8	49.4±17.7	NS
Glycine "	-3.3±10	-.3±4.1	NS	-21.8±14.4	-17.2±7.9	NS
Alanine "	2.5±10.5	-.9±8.6	NS	-47.6±14.9	-42.5±12.7	NS
Citrulline "						
α-amino-n-butyrate "	-2.7±.3	2.3±4.3	NS	-3.3±2.1	-8.8±2.7	.1
Valine "	-21.1±1	-10.4±.4	.02	-10.4±10.6	-16.1±9.9	NS
Cystine "	1.6±1.6	-1.4±7.0	NS	-.98±2.49	-4.8±6.5	NS
Methionine "						
Isoleucine "	-5.8±3.7	-9.7±2.5	NS	-5.88±3.5	-3.59±5.6	NS
Leucine "	-21.3±6.8	-28.5±15.3	NS	-16.5±9.6	-12.7±2.4	NS
Tyrosine "	-.6±.6	-2.6±4.2	NS	-5.9±4.6	-4.2±1.7	NS
Phenylalanine "	3.6±1.2	-.4±4.8	NS	-7.04±3.75	-7.74±4.82	NS
Ornithine "	3.2±1	9.8±4.9	NS	4.16±3.47	2.90±2.9	NS
Lysine "	-10.2±7.9	22.6±41.7	NS	-8.08±7.79	-10.21±16.77	NS
Histidine "	5±1.6	22.5±10.5	NS	.49±3.52	4.2±10.7	NS
3-Methyl histidine "						
Arginine "	4.3±2.8	-8.4±8	NS	-2.9±1.4	15.8±4.02	NS
Glutamine (enz) "	-5.1±7.4	8.9±23.8	NS	21.9±1.4	27.5±20.1	NS
Glutamic acid (enz) "	-6.6±1.5	-2.2±4.5	NS	-8.1±21.3	-6.7±1.5	NS
Phosphoserine "	-2.7±7.2	10.7±9.1	NS	-11.8±5.0	-13.9±7.6	NS

Flux = Mean±SEM, μM/min

Ψ Pair t-test, 3 dogs; Probability that $Flux_{PA}$ does not differ from $Flux_{3rd\ Day}$.

fast averaged 146±114 μM/min as compared to 134±97 μM/min respectively. Alanine extraction did not change significantly. More aspartic acid was extracted after 3 days than after an overnight fast ($p=.05$). Proline was released rather than extracted ($p=.1$). β-Hydroxybutyrate production doubled after a 3 day fast. This difference was not significant if the raw values are used for comparison but reach significance ($p=.05$, single tail) if percent changes are used instead.

Maximal possible hepatic glucose produced from peripheral gluconeogenic amino acids and substrates averaged 49±9% during the postabsorptive state and 74±26% after a 3 day fast. This increment in glucose production from peripheral gluconeogenic substrates and amino acids is significant ($p=.05$, single tail) if percent changes are utilized instead of raw values.

Table X

Substrate Fluxes Across the Liver in the Postabsorptive and Briefly Fasted State

	Postabsorptive State	3 Day Fast	P
Glucose	-325±98	-253.3±130.5	NS
Lactate	161.2±31.2	95.9±9.5	.15
Pyruvate	24.9±3.8	14.3±1.3	.17
Lactate/Pyruvate Ratio			
α-Ketoglutarate uM/L	3.46±1.34	3.79±.61	NS
Citrate uM/L	-27.1±21.7	.13±.61	NS
β-Hydroxybutyrate uM/L	-51.5±16.7	-104.3±55.8	NS
Acetoacetate uM/L	-37.2±9.9	-47.9±24.5	NS
β-OH/AcAc Ratio			
Glycerol uM/L	38.2±10.7	27.5±21.1	NS
Ammonia (blood) uM/L	76.2±19.6	55.6±10.9	NS
Taurine uM/L	.28±13.9	-27.8±29.8	NS
Aspartic Acid "	-1.18±2.42	3.26±1.57	.05
Threonine "	7.62±14.12	30.55±28.96	NS
Serine "	7.93±5.12	-.55±13.39	NS
Asparagine "	2.99±2.59	-2.33±6.71	NS
Proline "	30.65±10.44	-7.24±2.29	.1
Glycine "	18.5±11.1	16.82±4.66	NS
Alanine "	50.3±18.0	41.6±7.24	NS
Citrulline "			
α-amino-n-butyrate "	.571±2.209	11.1±6.9	NS
Valine "	-10.7±10.7	5.7±9.9	NS
Cystine "	2.6±4.1	3.4±3.3	NS
Methionine "			NS
Isoleucine "	.079 ±7.202	-6.1±5	NS
Leucine "	-4.8±8.2	-15.8±14.2	NS
Tyrosine "	5.3±4.0	1.6±2.7	NS
Phenylalanine "	10.7±4.6	7.3±3	NS
Ornithine "	-.96±4.48	6.97±4.7	NS
Lysine "	-2.07±13.27	32.8±25.2	NS
Histidine "	4.52±5.13	18.3±8.6	NS
3-Methyl histidine "			
Arginine "	7.28±3.4	7.42±10.52	NS
Glutamine (enz) "	-27±15.5	-18.6±8.1	NS
Glutamic acid (enz) "	1.5±1.7	4.7±6	NS
Phosphoserine "	9.14±4.8	5.5±5.2	NS

Mean ± SEM, μM/min

P = Paired t-test, n=3

Discussion

The simultaneous assessment of interorgan fuel hormone metabolism has not been previously characterized in either man or dog. The role of the kidney, the non-hepatic splanchnic bed and the hindleg in the adaptation to a brief fast have not been previously studied. Therefore, it was the purpose of this project to characterize the metabolism of the fasting dog, by means of simultaneous measurements across all the major tissues of the body, in order to better understand its relationship to the metabolism of the fasting man.

Body weight changes

Body weight consistently fell by 8% after a 3 day fast. This weight change is consistent with the observations of Lemieux (1968) and Brady (1977). Lemieux (1968) found that his dogs lost an average of 200 grams of body weight daily. Extracellular and intracellular volumes did not change in his 12 day fasted dogs and of the 200 grams lost, 82 grams were from fat and 104 grams from fat free protoplasm. Natriuresis accompanied by a loss of extracellular and intracellular volumes rather than the loss of fat is considered the mechanism which the initial weight loss at the beginning of a fast is accounted (Saudek, 1976, De Haven, 1980). This natriuresis is thought to be mediated by the fall in insulin concentrations. Felig recently showed that a hypocaloric diet rich in carbohydrates prevented the abrupt urinary sodium excretion observed with hypocaloric carbohydrate poor diets (De Haven, 1980). Urine analysis and body composition estimates were not done in this study, but would have been useful in determining the composition of the weight loss throughout the fast.

Accuracy of Blood Flow Measurements

Blood flow determinations were done across the splanchnic bed, kidney and hindleg in order to quantitate the movement of substrates and amino acids from one organ to the other. Splanchnic blood flow was calculated by the continuous infusion of Indocyanine green by the method of Ketterer (1960). Teranaka (1977), using electromagnetic measurements of blood flow across the splanchnic bed in healthy normovolemic, normotensive dogs found that the accuracy of hepatic blood flow determinations with Indocyanine green was 98%. Since the splanchnic bed blood supply arises from 2 sources, the portal vein and the hepatic artery, a large error is introduced by the uncertainty of the proportions of total hepatic blood flow carried by the hepatic artery and portal vein. In unanesthesized animals, Blalock (1936) reported the percentage of arterial to total hepatic flow to range from 12.6 to 24.5% with a mean of 19.5%. Observations by Katz (1969) with the simultaneous use of BSP hepatic clearance and PAH dilution technique in awake dogs showed that the percentabe of arterial to total hepatic artery ranges from 5 to 31% with a mean of 15%. A portal flow of 80% of total hepatic flow, based on the values of Blalock (1936) and Katz (1969) has been used for the calculations reported in Tables IX, X, and Figures 1 to 3. Splanchnic blood flow was successfully measured on all four dogs during the postabsorptive state but only in 3 after a 3 day fast. The values presented here are the result of pair t test analysis on 3 dogs, unless stated otherwise.

Splanchnic blood flow in the postabsorptive state ranged from .799 to 1.1506 liters/minute with a mean of .9469±.1052 liters/minute (n=3) or .9328±.0757 liters/minute (n=4) which translates to 44.1±4.9 cc/kg/min (n=3) or 42.4±2.8 cc/kg/min (n=4). These values are similar to those reported by Katz (1969), Teranaka (1977) and Shoemaker (1960). After 3

days of starvation splanchnic blood flow averages 1.2531±.5773 liters/minute (n=3) or 49.±26 cc/kg/minute which are values similar to those reported by Keller (1978) in awake 48 hour fasted dogs (51.4±5.7 ml/kg/min). --

Renal blood flow was calculated with the continuous PAH infusion technique by the method of Harvey (1962). Renal blood flow was determined in all 4 dogs after an overnight and a 3 day fast. Renal vein blood was immediately centrifuged at 4°C to separate the cells from the plasma; thereby, minimizing the diffusion of PAH from erythrocytes to plasma (Harvey, 1962).

After an overnight fast renal blood flow was .3798±.0445 liters/minute or 17.3±2.2 cc/kg/minute. These are values similar to those reported by Houck (1948) but larger than those of Churchill (1970) and Pitts (1972) in anesthesized animals. After 3 days of starvation (water ad lib) renal blood flow fell, but this was not significant. The blood flow after 3 days of starvation was similar to those reported by Churchill (1970) in his 60 hour fasted dogs. The extraction fraction for PAH across the kidney was 82% which was similar to the values reported by Churchill (1970), Horster (1971) and Pitts (1968).

Hindleg blood flow was measured with the capacitance plethysmograph in only 3 dogs in the postabsorptive state and in 4 in the briefly fasted state (Figar, 1959, Aoki Appendix 1). During the postabsorptive state the blood flow per 100 cc of hindleg was 6.5±2.1/minute and increased to 8.92±2.46 cc/minute (p=.05, single tail, n=3) after 3 days of fasting. This increment in hindleg blood flow has previously been reported in man by Pozefsky (1969) and Finley (1980). Pozefsky (1969) found that this increment in blood flow was mainly directed at muscle. Our postabsorptive state values are in agreement with what is expected if the muscle, bone

and skin under the plethysmograph cuff, have received the flow which each of this tissues receives according to the findings of Rogers (1972). Applying Rogers' values to the relative composition of the hindleg, as determined by dissection (Appendix 2), the predicted blood flow is 6.9 cc/minute/100 ml of hindleg as compared to the obtained 6.5±2.06 cc/minute/100 ml of hindleg with the capacitance plethysmograph.

Dissection of 4 dog hindlegs showed that the hindleg comprises 8% of the dog's total body weight. The hindleg was 60% muscle by volume and weight. The volume under the capacitance plethysmograph was 75% muscle, 20% skin and 5% bone. This difference between the relative composition of the hindleg and the volume where the blood flow measurements were determined could have lead us to overestimate by at least 10-15% the entire blood flow to the hindleg (flow/100 cc of hindleg X the total volume of the hindleg). After an overnight fast the hindleg received 108.7 cc of blood/minute. These values are similar to those obtained by Spence (1972) and Cronenwelt (1978) with an electromagnetic probe.

Carbohydrate Metabolism

Blood glucose concentrations did not change with fasting. Cahill (1966) in man, and Brady (1977) and Belo (1977) in dogs, found a fall in glucose concentration after 48 to 72 hours of food deprivation. Lemieux (1968)however, found that blood glucose concentrations remained stable with fasting. The relative composition of the diet preceding the fast could have been responsible for this difference. Foss (1979), in rats fed a high protein low carbohydrate diet, found that glucose concentrations did not decline after a 24 hour fast while they fell significantly in rats fed a high carbohydrate diet. The diet consumed by our dogs prior to the fast contained only 7% carbohydrate on a metabolizable energy basis while the subjects studied by Cahill and Brady consumed

diets which contained up to 24% carbohydrate.

Hepatic glucose production did not change significantly after a 3 day fast. Hepatic glucose output in the postabsorptive state was 12.8 μM/liter per kilogram of body weight or 15.1 μM/kg/minute which is comparable with the rates reported by Cherrington (1976) and Elwyn (1968) in postabsorptive anesthesized and awake dogs respectively. After 3 days of food deprivation hepatic glucose production fell by 15-18% but this change was not significant. Hepatic glucose production after fasting was 10.5 μM/liter/kg or 12.8 μM/kg/minute which is similar to the values reported by Keller (1977). The dogs used by Keller (1977) and Cherrington (1976) were anesthesized with pentobarbital so it is possible that this agent might have an insignificant effect on glucose production by the liver.

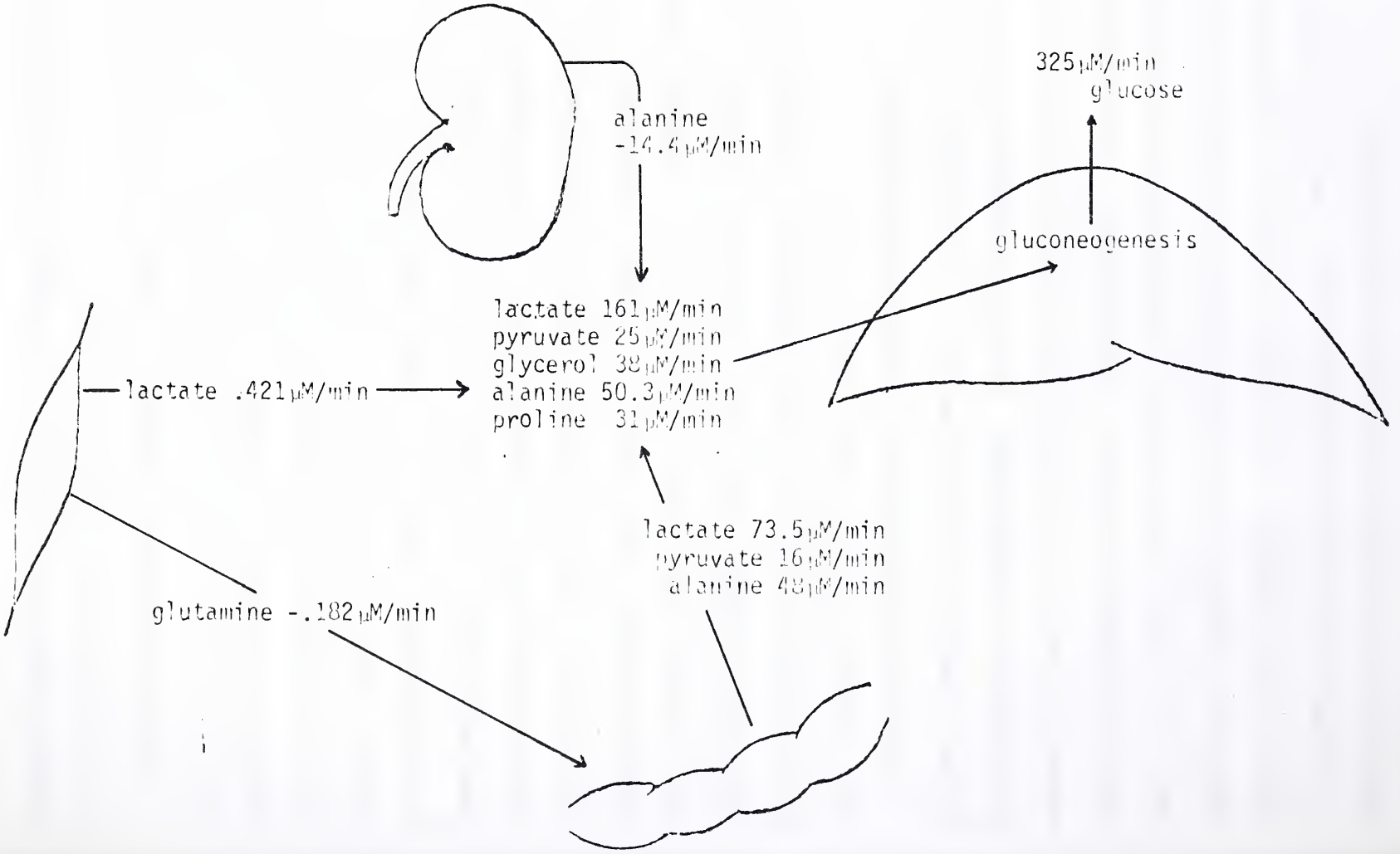
The hepatic and non-hepatic splanchnic bed fluxes, which were obtained in only 3 of the 4 dogs, reveal the possible source of the gluconeogenic precursors for glucose hepatic production. Gluconeogenic precursors are defined as lactate, pyruvate, α-ketoglutarate, citrate, glycerol and all the amino acids, except for leucine. Lactate, pyruvate, the Tricarboxylic Acid Cycle intermediates, and glycerol and α-amino acids are essentially 3 or 4 carbon substrates and their sum divided by 2 gives an estimate of the amount of glucose that could be formed from these precursors if all were converted into hexose. Leucine, a ketogenic amino acid, is not oxidized by the liver (Elwyn, 1968). The extraction of gluconeogenic precursors after an overnight fast could have accounted for 49% of the glucose produced by the liver if all the extracted precursors had been converted into glucose. The other 51% of the hepatic glucose production could have arisen from hepatic glycogenolysis or gluconeogenesis from liver proteolysis. After a 24 hour fast the dog liver

contains an average glycogen glucosyl content of 235±19 μM/kg of wet liver or 127 mm glycosyl units (Kobryn, 1978). In 2 of the 3 dogs with calculated splanchnic bed fluxes after an overnight fast the liver extracted leucine. This suggests net hepatic protein synthesis, since leucine is not oxidized by the liver (Elwyn, 1968). The remaining dog showed a net release of leucine as well as other amino acids, suggesting marked liver proteolysis. Sixty seven percent of glucose produced in the liver of that dog could have arisen from intra-hepatic sources. This dog also had the highest rate of hepatic ketogenesis. This marked liver proteolysis and ketogenesis all indicate that this dog had the lowest physiologically active insulin concentration (Aoki, 1979).

Figures 1 to 3 show the interorgan fuel interchange between the liver, non-hepatic splanchnic bed, kidney and skeletal muscle during the postabsorptive and briefly fasted states. Fluxes across the hindleg are assumed to represent the metabolism of skeletal muscle. Bone and skin, which make up 18 and 23% of the hindleg respectively, are assumed to be metabolically inactive (Appendix 2). The fluxes quoted for skeletal muscle will be underestimations since the arteriovenous differences across muscle will be diluted by blood coming from bone and skin.

Figure 1 shows the interactions of the liver, NHSB, kidney and muscle in the movement of gluconeogenic precursors to the liver in the post-absorptive state. It is assumed that all the precursors extracted are converted into glucose. Chiasson (1977) has shown that not all the gluconeogenic precursors extracted by the liver are converted into glucose and that their relative conversion into glucose increases with the duration of the fast. Therefore, our estimates on the relative contribution from gluconeogenic precursors are maximum values. The combination of both arteriovenous differences, blood flow and radioactive tracers

Figure 1: The Interaction of the Liver, Non-Hepatic Splanchnic Bed, Kidney and Muscle in the Movement of Gluconeogenic Precursors in the Postabsorptive State.



techniques would have given us a more complete understanding of the fate of the extracted gluconeogenic precursors.

Hepatic extraction of lactate could have accounted for 25% of the glucose produced by the liver. Forty seven percent of the lactate extracted was released by the NHSB. The rest came from the other glycolytic tissues of the body such as the blood and bone marrow cellular elements, muscle and renal medulla. Lactate was significantly produced by the hindleg at a rate of .421±.0258 μM/minute/100 cc of hindleg. There was no significant lactate production by the kidney which means that the renal cortical consumption equals the renal medullary production (Neith, 1964). Lactate consumption surpassed production since arterial lactate levels fell significantly as the fast progressed. This fall in lactate concentration with fasting is consistent with the findings of Brady (1977) in dogs. Aoki (1975) in man and Belo (1977) in the dog failed to see any change in lactate concentrations with fasting. The reason for the different observations is unknown.

Pyruvate extraction by the liver was responsible for 4% of the glucose released. Sixty four percent of that pyruvate originated within the non-hepatic splanchnic bed. Neither the kidney nor skeletal muscle produced pyruvate in the postabsorptive state. The source of the remaining pyruvate is unknown. Pyruvate arterial concentrations remained stable with fasting indicating that pyruvate consumption equalized production. Glycerol extraction by the liver accounted for 6% of the hepatic glucose produced. This value is identical to the ones found by Hall (1976) in dogs with radioactive tracers. Arterial glycerol concentrations did not change with fasting (Owen, 1969).

Figure 1 shows that alanine was the main gluconeogenic amino acid extracted by the liver. Eight percent of hepatic glucose production could have arisen from this amino acid. Ninety six percent of the alanine extracted by the liver was released by the portal drained bed. Alanine production by the NHSB accounted for 23% of all the gluconeogenic precursors released which is consistent with the findings of Aikawa (1973) and Matsatuka (1973) in the rat, Windmuller (1974, 1977) in tne dog and rat, Weber (1977) and Vaidyanath (1978) in the dog, and Felig (1971, 1973) in man. Wahren and Felig (1971, 1973) found that up to 50% of the alanine extracted by the liver could have come from this bed. Our findings show that 96% of the alanine extracted by the liver came from the NHSB. Still the rates of production found by Felig (41 μM/minute) and us (48 μM/minute) are similar. In addition to alanine, proline was also extracted by the liver and accounted for 5% of the glucose produced. There was no net splanchnic extraction of alanine in tne postabsorptive state. This is inconsistent with the findings of Felig (1969), Garber (1974), Aoki (1976) and Chiasson (1979) in man. There is no available explanation for this inconsistency other than this might be a species difference. Alanine was also produced by the kidney which is consistent with the findings of Shalhoubs (1973) in acidotic dogs and Owen (1963) in normal man. The kidney and NHSB alanine production was slightly more than that extracted by the liver and this might explain why the alanine arterial concentrations did not fall significantly with fasting.

There was no significant alanine production by the hindleg. Felig (1970) and Pozefsky (1969) have shown that alanine makes up 30% of the amino acid efflux from human forearm muscle. Most of this alanine is

derived by the transamination of pyruvate which is produced by the glycolysis of glucose. This is known as the alanine-glucose cycle (Felig, 1973a). Recently Aoki and Finley (1980) have challenged the importance of alanine as a carrier of glucose derived 3 carbon skeletons from muscle to periphery. They found that alanine made up only 16% of the nitrogen released into whole blood by muscle during carbohydrate loading. Glutamine accounted for 58% of the nitrogen efflux. Glutamine, not alanine, was the only amino acid which tended to be released (-.182±.029 μM/minute/100 cc of hindleg) from the hindleg during the postabsorptive state. The origin of this glutamine is not known since glucose was not significantly extracted by the hindleg. Transamination of otner amino acids into glutamine could have accounted for the efflux of this amino acid (Chang, 1978b).

Glutamine was the only amino acid which was extracted by the non-hepatic splanchnic bed in the postabsorptive state. This is consistent with the findings of Aikawa (1973), Matsutaka (1973), Windmuller (1974, 1977) and Felig (1971, 1974). The kidneys of 2 out of the 3 dogs studied extracted glutamine (23.9 and 17.9 μM/minute) while the kidney of the third dog produced this amino acid (-11.7 μM/minute) making arterial-venous glutamine differences across this organ not significantly different from zero. Ammonia was produced by both the kidney and non-hepatic splanchnic bed. The non-hepatic splanchnic bed produced more ammonia than the kidney. The role and origin of the ammonia produced by this bed is still controversial. Windmuller (1974) found that ureolytic intestinal bacterias were the main origin of this ammonia. Matsutaka (1973) however, found that the ammoniagenesis induced by glutamine loading was the same in germ free and normal rats. Therefore, the role of glutamine uptake and ammonia production by this bed is not clear at

this time. It is possible that glutamine serves as the carrier of amino groups derived from the oxidation of BCAA in muscle to the splanchnic bed without permitting the accumulation of ammonia in the systemic circulation. Ammonia is then released into the portal circulation where it is readily extracted by the liver and converted into urea. Forty three percent of the blood ammonia was extracted by the liver in one pass leading to no net change in ammonia concentration across the splanchnic bed. Confirming the hypothesis that glutamine is the main carrier of BCAA derived amino groups Aoki (1980) found that glutamine efflux from human muscle increased significantly after a leucine meal while alanine efflux increased only slightly. The earlier studies in which the present theory of the alanine-glucose cycle is based involved the measurement of amino acid fluxes in plasma rather than in whole blood. In addition many of the earlier studies also lacked a suitable, reproducible technique for glutamine analysis. These 2 considerations may explain the difference in the findings of Finley, Aoki and the present study with those of the past. This question of the role of glutamine and alanine in the transfer of carbon skeletons for gluconeogenesis and amino groups from the periphery to the liver will require further work.

In summary, during the postabsorptive state, 48% of the glucose produced by the liver could have been the product of gluconeogenesis from peripheral precursors. Of the extracted gluconeogenic precursors 65% were produced by the non-hepatic splanchnic bed, a tissue which has not been adequately studied in man because of technical problems involved in obtaining samples from this bed.

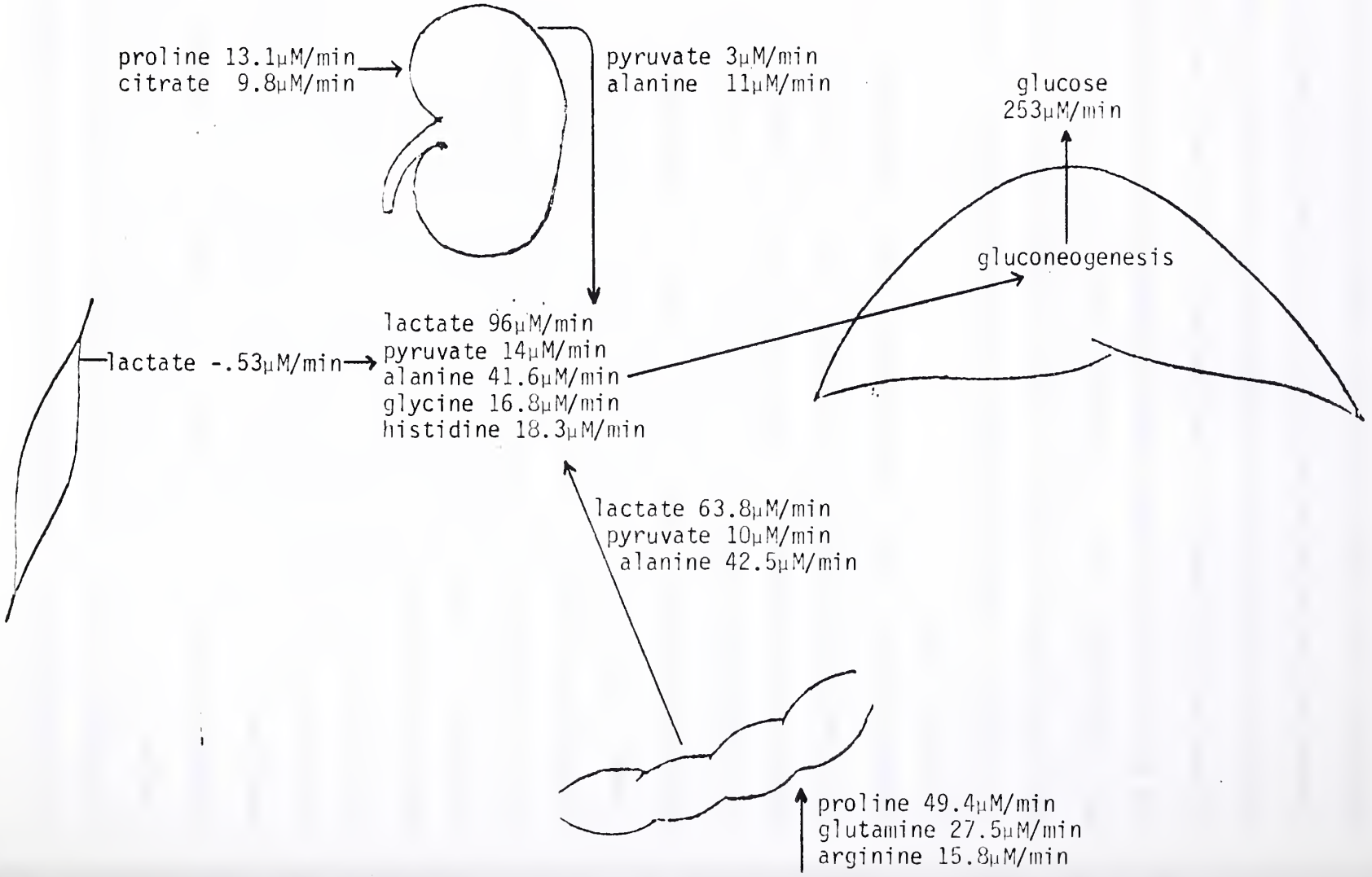
Two of the 3 dogs with splanchnic blood flow determinations experienced net glucose production by the total splanchnic bed (-143.9

-265μM/min). The other dog (CA) whose splanchnic bed consumed glucose rather than produced it had the highest levels of arterial insulin concentration as well as the lowest rate of hepatic ketogenesis. The liver of this dog produced more glucose (-480.9 μM/minute) than the other dogs but the positive glucose balance across the splanchnic bed was due to the highest rate of glucose consumption (492.4 μM/minute) by the non-hepatic splanchnic bed. Shoemaker (1960) showed that the uptake of glucose by non-hepatic splanchnic bed was more sensitive to elevated insulin concentrations than the rate of glucose hepatic production and that the fall in splanchnic glucose output following insulin administration was secondary to an increased glucose uptake by the non-hepatic splanchnic bed.

Net glucose production by the splanchnic bed was 114.8±74.2 μM/minute (5.3±3.4 μM/kg/minute) which was not statistically significant from zero. The non-hepatic splanchnic bed consumed 215±144 μM/minute (range= .00639-.4924) or 66% of the hepatic glucose produced. Neither the kidney (Dies, 1970) nor the hindleg produced or consumed glucose. The fate of the glucose not extracted by the non-hepatic splanchnic bed is unknown. In order to gain a better understanding of the fate of the glucose produced the measurement of fluxes across the brain should be included in the animal preparation as well as the concomitant use of labeled glucose and its precursors.

Figure 2 shows a schematic representation of interorgan fuel exchange after 3 days of starvation. Glucose production by the liver was 15% less than that found in the postabsorptive state but this difference was not significant. Glucose production by the liver was 253±130 μM/minute or 12.8 μM/kg/minute. These averages are very similar to those reported by Keller (1977) and Cherrington (1976). As a group 64% of the glucose

Figure 2: The Interaction of the Liver, Non-Hepatic Splanchnic Bed, Kidney and Muscle in the Movement of Gluconeogenic Precursors After a Brief Fast



arising from hepatic gluconeogenesis can be attributed to hepatic extraction and conversion of such peripheral gluconeogenic precursors. However, in 2 of the 3 dogs studied, hepatic extraction and conversion of these precursors can account for all the glucose produced. The remaining dog's liver extracted a smaller amount of these precursors, and in addition released large amounts of leucine (-43 μM/minute). This observation suggests that the hepatic glucose produced in this dog (WA) came from gluconeogenesis from precursors derived from intrahepatic proteolysis. Hepatic glycogenolysis could also account for this glucose but this is improbable since the hepatic glycogen reserves are usually significantly depleted, if not exhausted, after 24 hours of fasting (Swenson, 1946, Kobryn, 1978). This same dog had the highest rate of 1) hepatic ketogenesis (301 μM/minute) and 2) hindleg efflux of glutamine (-.416 μM/minute) lysine (-.371 μM/minute), valine (-.208 μM/minute) and leucine (-.190 μM/minute). These observations are consistent with a pronounced physiologically insulinopenic state. The arterial insulin concentration was lower in this dog than that in the other animals (see Table IIb)

Figure 2 shows that lactate extraction by the liver accounted for 19% of the glucose produced. Lactate's hepatic extraction fraction did not change after 3 days of food deprivation. The NHSB continued to be the main source of lactate, producing 66% of that which was extracted. Lactate was also produced by the hindleg at a rate which was not significantly different from the postabsorptive state fluxes. Hepatic pyruvate extraction accounted for 3% of the glucose produced while the NHSB and kidney produced 71% and 21% respectively of the pyruvate extracted.

Figure 2 shows that alanine accounted for 8% of hepatic glucose production. The NHSB and kidney were the main sources of alanine while the hindleg again failed to release alanine significantly. Alanine production by the NHSB equalled alanine extraction by the liver. The kidney produced alanine at a rate similar to that of an overnight fast. Since there was net alanine production the arterial alanine concentrations did not change significantly after 3 days of starvation (Felig, 1969). Chiasson (1979) in man, using labeled ^{14}C alanine and arterio hepatic vein alanine concentration differences, found that alanine production by the NHSB falls to insignificant amounts after 48 hours of fasting. He also found a significant fall in arterial alanine concentration. These findings, which did not include arterio-portal measurements, are not consistent with our observations. The lack of alanine production by his human subjects could be responsible for the fall in the arterial alanine concentration. Felig (1969) using 2 sets of subjects failed to show that alanine concentrations were lower after a brief fast than after an overnight fast. Alanine metabolism within the splanchnic bed will require further study in order to explain the inconsistency between our results and those of Felig (1969) with those of Chiasson (1979). Along with alanine production, the NHSB produced α-amino butyrate and glutamate which were not significantly extracted by the liver.

Arginine, glutamine and proline were extracted but not significantly by the non-hepatic splanchnic bed (due to great variability in values; i.e., in the case of glutamine: 5.22 - 67.7 μM/minute were extracted by the NHSB). Glutamine was also extracted by the kidney at a rate no different than that measured in the postabsorptive state. Ammonia production by the NHSB fell by 59% with fasting but this change was not significant ($p=.1$) while ammonia excretion by the kidney fell by

25% (p=.05, single tail). The fall in urinary ammonia excretion with fasting was previously described by Lemieux (1968) and Churchill (1970). Proline was also extracted by the kidney after a 3 day fast which is similar to the findings of Shalhoub (1963) in his acidotic dogs. Along with the extraction of proline and glutamine by the kidney, serine was produced (Pitts, 1972).

Urinary nitrogen excretion fell with fasting. This is consistent with the findings of Lemieux (1968) in his 12 day fasted dogs. From the above discussion it is apparent that gluconeogenesis from amino acids increased after 3 days of starvation. Therefore, it was expected that nitrogen excretion would initially increase with fasting. However, the dogs had been placed on a very high protein diet for at least 2 weeks prior to the fast so that the nitrogen excretion after an overnight fast represented the nitrogen consumed the night before. Thus the nitrogen excreted in the postabsorptive state does not reflect the rate of gluconeogenesis from endogenous amino acids. This explains the marked fall in urinary nitrogen excretion following the continuation of the fast. The nitrogen excretion rate after the second day of the fast was significantly decreased from postabsorptive values (2.83±.3 mg/minute vs 8.9±1.2 mg/minute). However, the urinary nitrogen excretion rate after 3 days of starvation (3.3±.4 mg) was 8% higher than that after 2 days of starvation (p=.05). If we analyze the urinary data on only those dogs whose splanchnic fluxes were calculated, and in which an increased rate of amino acid derived gluconeogenesis was observed (n=3), the increase in urinary nitrogen excretion per minute was 27% (p<.01) in the third day of the fast as compared to the second day (3.57±.41 vs 2.61±.32 mg/minute). Thus, the increased rate of nitrogen excretion during the third day of the fast represents an increment in protein derived gluconeo-

genesis. An undetermined error is introduced by extrapolating this data to rates of amino acid derived gluconeogenesis because the values quoted here are based on urine collections which spanned only 4 to 6 hours. Therefore, 24 hour urine collections, using metabolic cages, should be performed and should provide a better understanding of net nitrogen balance, amino acid derived gluconeogenesis and protein sparing.

Ketone Body Metabolism

Arterial ketone levels after an overnight fast were 300% higher than the values reported by Basso (1970), Brady (1977) and Werner (1971). As expected, the arterial ketone concentration increased by 180% after 3 days of food deprivation ($p=.05$, single tail). The arterial ketone levels after fasting were slightly higher than those reported by Keller (1973), Vaidyanath (1978) and Brady (1977) after 2 to 7 days of starvation.

Figure 3 depicts the interorgan ketone exchange in the postabsorptive and briefly fasted states. In the postabsorptive state ketone production, as assessed by arteriovenous differences across the liver ($n=4$), averaged -80.1 μM/liter or 3.6 μM/liter/kg of body weight. Hepatic ketone fluxes ($n=3$) revealed a ketone production rate of 88.7±26 μM/minute or 4.14±1.16 μM/kg/minute ($p=.05$, single tail). This contrasts with the findings of Basso (1970) who found a rate of ketone production of 1.03±.25 μM/kg/minute. The different rates of ketogenesis found by us explains why our ketone arterial concentrations are higher. The differences in ketogenesis are probably attributable, in part, to the varying glucose, and hence insulin, levels. Basso's non diabetic dogs had an arterial glucose concentration of 106.5±3.9 mg/100 ml of blood as compared to the 87±3 mg/100 cc of blood found in this study.

Figure 3a Shows the Interaction of the Liver, Non-Hepatic Splanchnic Bed, Kidney and Muscle in the Metabolism of Ketone Bodies During the Postabsorptive State. See Text for Explanation. Values are expressed as μM/min.

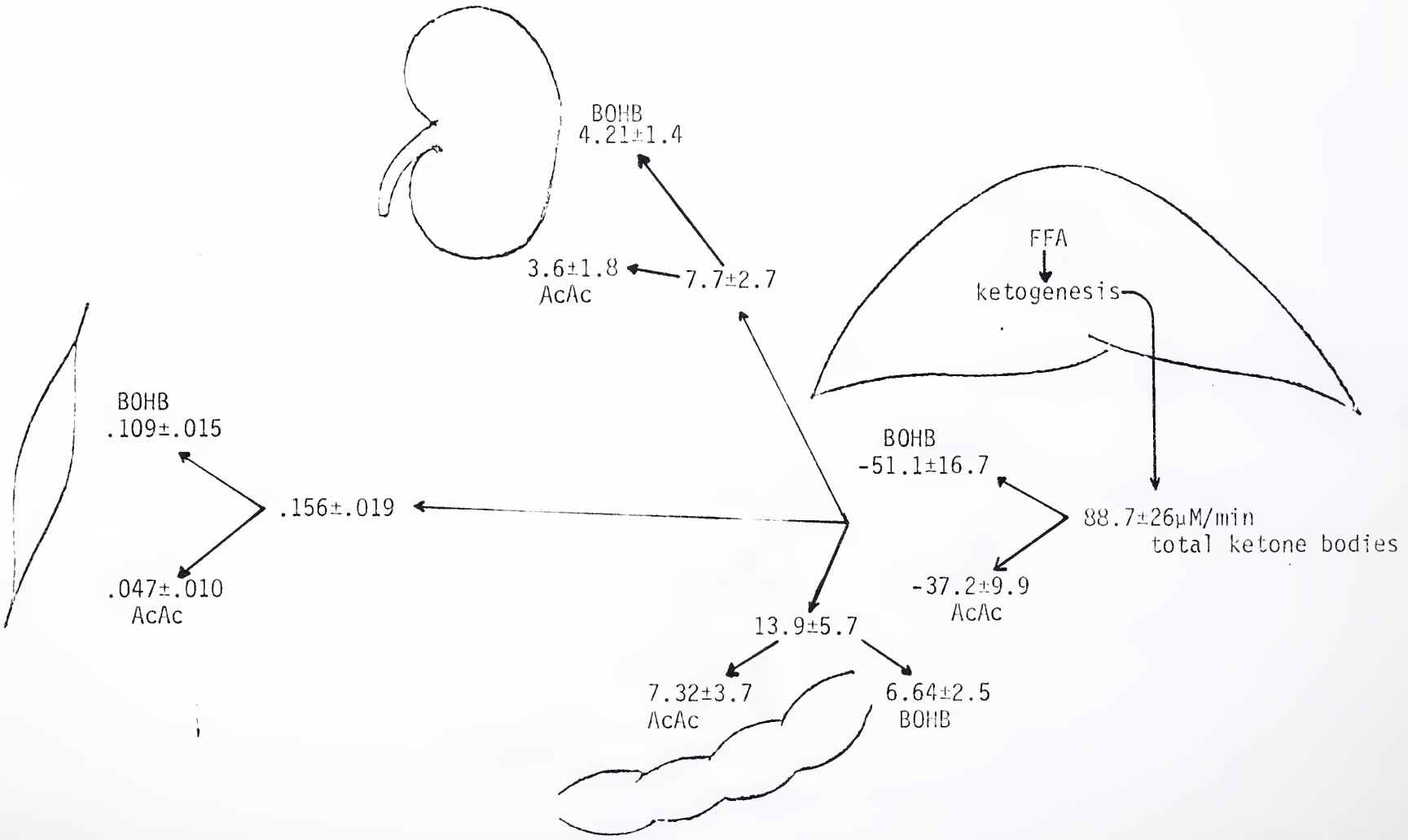
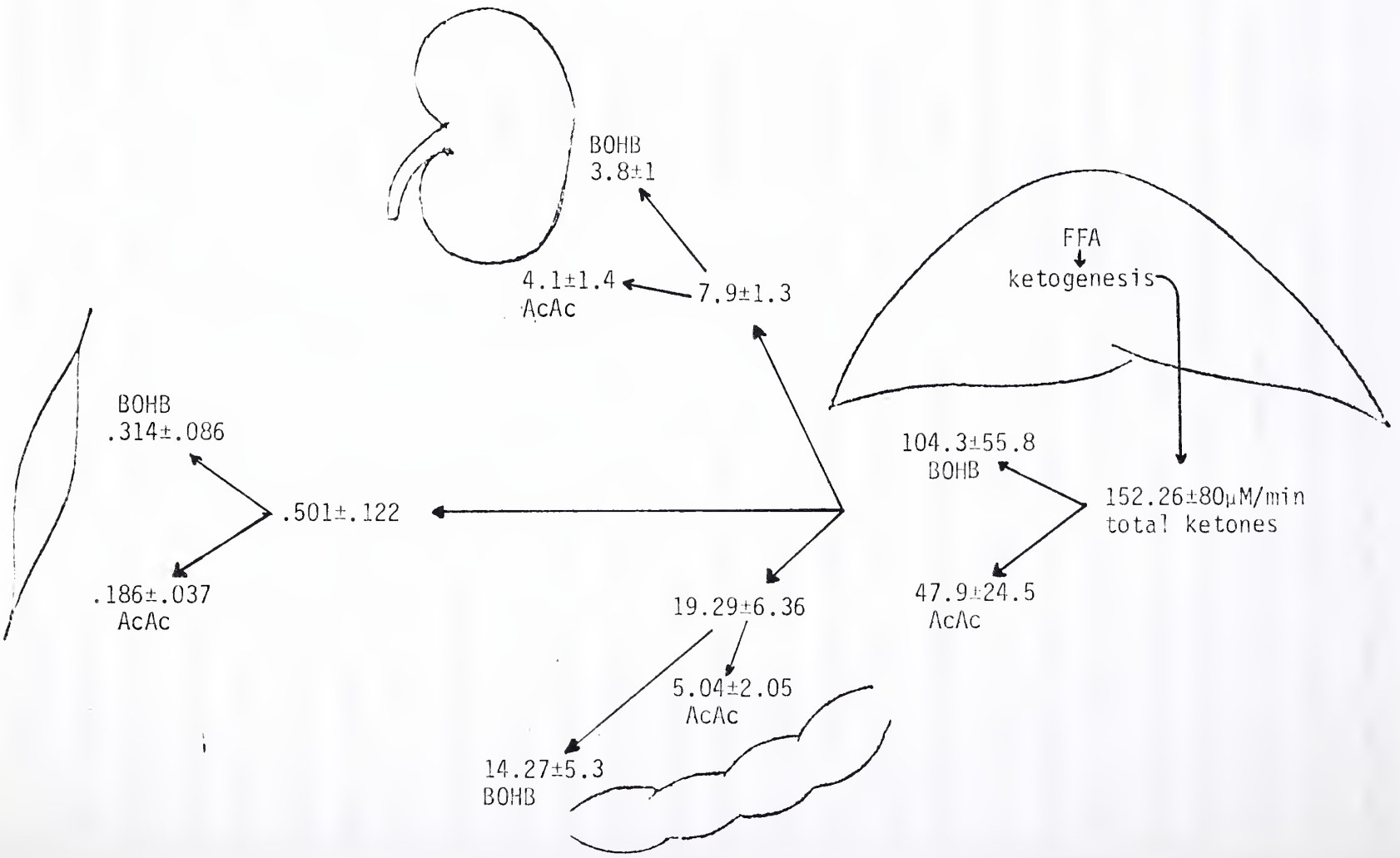


Figure 3b Shows the Ketone Body Metabolism After 3 Days of Starvation. Values are expressed as μM/min.



After 3 days of starvation ketone production increased by 175% to 128.1 μM/liter or 6.3 μM/liter/kg of body weight when arteriovenous differences are averaged (n=4). In the 3 dogs whose splanchnic blood flows were successfully measured, the rate of ketogenesis increased in 2 out of the 3 dogs by 191% (p=.05, single tail). The other dog (CA) behaved differently. The rate of ketogenesis in this animal fell rather than increased. After 3 days of starvation, the insulin levels were similar in all 3 dogs; however, the changes in circulating insulin levels in CA differed from the other three animals. Initially, insulin levels increased unlike that of the other 3 dogs, from 22 μU/ml in the postabsorptive state to 35 μU/ml after 2 days of starvation and then fell to levels similar to those of the other dogs. The fact that free fatty acid arterial concentrations were the lowest of all in this dog and that leucine was avidly extracted by the liver, suggesting liver protein synthesis, suggests that this dog consumed some food during the fast. The rate of hepatic production excluding this dog was 11.3 μM/kg/minute (7.7±4.1 μM/kg/minute if all 3 dogs are averaged), a rate similar to those reported by Keller (1978) in his 48 hour fasted dogs.

The arterial ketone levels in dogs are insignificant when compared to those of man during a fast (Crandall, 1946, Brady, 1977, Lemieux, 1968). Since ketonemia is considered an important factor in the complex process by which man is able to spare protein during a fast the study of ketone metabolism in the dog is important if the response to fasting in these 2 species is to be compared. The rates of ketogenesis found in our study are similar to those found by Keller (1978) and higher than those found by Reichard (1974) in briefly fasted man. The rates of hepatic ketogenesis in the dog continue to be controversial since

Keller in 1977, found hepatic ketogenesis at a rate of 1.78 μM/kg/minute after 3 days of starvation. These values are very similar to those reported by Basso (1970) in his 18 hour fasted animals. The common denominator between these two reports is that in both cases the dogs were anesthesized with sodium pentobarbitol. Keller (1978) repeated his studies in awake dogs a year later and found values 5 fold higher and similar to those found by us. This difference in ketogenesis is probably secondary to the use of pentobarbital. Pentobarbital is known to stimulate insulin secretion (Hellman, 1977). Ketogenesis is particularly sensitive to changes in insulin concentration; therefore, even a small increment in this hormone will depress ketogenesis without affecting the arterial concentrations of free fatty acids or glucose (Aoki, 1979).

The rate of hepatic ketogenesis in the dog after 3 days of starvation is very similar to those of man (Garber, 1974, Reichard, 1974, Grey, 1975, Owen, 1971). As mentioned above, the degree of ketonemia is markedly different between the two species. Brady (1977) and Lemieux (1968) believe that this difference is probably due to differences in the rate of ketone oxidation. Along with the increased rate of hepatic ketogenesis after a 3 day fast our findings show an increment in the rate of ketone metabolism by the different organs of the dog.

Figure 3 shows the metabolism of ketone bodies after an overnight and 3 day fast. Ketone bodies were extracted 312% ($p=.025$, single tail) more by the dog hindleg after 3 days of starvation than in the postabsorptive state. This difference is particularly significant for acetoacetate (AcAc). Acetoacetate consumption by muscle increased by 400% after a 3 day fast. Total ketone consumption by the postabsorptive dog hindleg was .156±.019 μM/minute per 100 ml of hindleg as compared to

.501±.122 μM/minute/100 ml after 3 days of food deprivation. This marked change in ketone extraction by the hindleg, which has not been previously reported in the dog, is similar to the 500% increment reported by Owen (1971) in obese 3 day fasted humans.

The non-hepatic splanchnic bed is very much involved in the clearance of ketone bodies. β-hydroxybutyrate (BOHB) was significantly extracted by the postabsorptive dog NHSB (p=.05). After 3 days of starvation BOHB arterio-portal concentration differences averaged 22±6.8 μM/liter (p=.025, single tail) as expected from the work of Vaidyanath (1978), who found an uptake of about 34 μM/liter of blood. The arterio-portal BOHB concentration differences were 244% larger after 3 days than after an overnight fast. When fluxes rather than arterioportal differences are analyzed (n=3) there was an increment of 146±24% (p=.05) in total ketone body consumption after a brief fast (13.96±5.75 μM/minute or .65±.27 μM/kg/minute vs. 19.31±6.37 μM/minute or .98±.32 μM/kg/minute). Thus, the non-hepatic splanchnic bed consumes 16% of the hepatic ketone production in both the postabsorptive and the briefly fasted state.

The kidney consumes ketones at a rate of 7.7±2.7 (or .35±.12 μM/kg/minute) and 7.9±1.3 (or .39±.06 μM/kg/minute) in the postabsorptive and briefly fasted states respectively. That is, the kidney consumed 34% and 5% of the ketones produced in the postabsorptive and briefly fasted state respectively. The kidney did not excrete ketones as measured by the nitroprusside reaction. β-hydroxybutyrate excretion would have been missed by this method but since urinary ammonia excretion fell, it would seem most unlikely that the β-hydroxybutyric acid excretion increased (Pitts, 1972, Cahill, 1975a).

Branched Chain Amino Acids

Table II shows the arterial concentration of the branched chain amino acids (BCAA) after an overnight and a 3 day fast. The concentrations of leucine and valine fell while that of isoleucine did not change. This initial decrement in the arterial concentrations of the BCAA contrasts with the findings of Felig (1969) who found that the concentrations of the BCAA increased early in the fasting period but fell below postabsorptive concentrations after 2 to 3 weeks of starvation. Brady (1977) found that after 1 day of fasting the BCAA levels in dogs fell (significantly only for isoleucine). He also found that the leucine and valine concentrations were significantly elevated after 7 days of food deprivation when compared to the first day of fasting but not when compared to the postabsorptive values. (Isoleucine was an exception, being significantly higher after 7 days of starvation when compared to either the postabsorptive state or after one day of starvation.) This discrepancy between our data and that of Felig and Brady may be due to the diet ingested by the study subjects prior to the initiation of the fast. The subjects studied by both Felig and Brady ate a carbohydrate rich diet while our dogs consumed a high protein, carbohydrate poor diet. Since the metabolism of individuals consuming carbohydrate poor diets is similar to that seen with prolonged fasting it is possible that the concentration of the BCAA fell along with that of the other amino acids as it was observed by Felig after 17 days of food deprivation.

The decrement in the concentrations of the BCAA with fasting was primarily due to a decreased efflux of these amino acids from the splanchnic bed. For example; valine efflux fell by 50% after 3 days of starvation (-21±1 to -10.4±.4μM/min; p=.02). A significant leucine release from the

splanchnic bed fell to insignificant amounts after 3 days of food deprivation. This observation is consistent with the findings of Sherwin (1978) who found that the fall in the BCAA concentration after a prolonged fast is due to a decrement in the plasma delivery rate of these amino acids. Glucagon is probably involved in the observed fall of these amino acids. This hormone is commonly known to increase liver proteolysis and BCAA efflux from perfused liver. The levels of glucagon fell significantly after 3 days of starvation and accompanied the fall in the efflux of the BCAA from the splanchnic bed.

Further experimentation with the use of different diets preceeding the fast, with a larger number of animals and longer fasts are necessary in order to understand the role of the BCAA on protein metabolism.

Regulatory Hormones

Substrate metabolism is influenced by many factors. These are: 1) the hormonal milieu, 2) the activity of the nervous system, 3) the diet and nutritional status of the individual, 4) the redox state of the cellular cytoplasm and mitochondria, 5) and the actual concentration of the substrates in the blood and tissues. For example, glucose is believed to partially autoregulate its metabolism at the liver (Bergman, 1977).

Insulin

The arterial insulin concentrations averaged 17±2 μU/ml in the postabsorptive state. These levels are similar to those reported by Jennings (1977) in his postabsorptive dogs. After 3 days of starvation the insulin concentration fell by 40% to 10±1 μU/ml ($p=.05$). This fall in insulin concentration is similar to that observed by Cahill (1966) in briefly fasted man. Unlike the findings of Cahill, who correlated the fall in insulin with that of glucose, the levels of glucose in our dogs did not

change with fasting. Therefore, the fall in insulin concentration is probably explained by the concominant fall in the concentration of amino acids. Amino acids are known to stimulate the secretion of insulin, but in omnivarous man, insulin is mainly modulated by glucose, which is the strongest physiological secretagogue (Lefebrve, 1972). The dog is different since it is a carnivore and therefore consumes very little dietary carbohydrate. Thus, from a teleologic point of view BCAA concentrations may play a relatively more important role in the dog with respect to pancreatic β-cell stimulation than in man. It is not possible from our data to conclusively show that the fall in BCAA is the main factor influencing the fall in insulin concentration. Studies addressing this question should be performed in the future.

Accompanying the fall in the level of insulin, the concentrations of FFA increased by 36% ($p=.01$). Ketogenesis, the most insulin sensitive reaction, increased along with the decrement in the concentration of this hormone.

Glucagon

The arterial concentration of glucagon in the postabsorptive state was 65±6 pg/ml which was half the concentration found by Cherrington (1976) in his pentobarbital anesthesized dogs. Chiasson (1980, personal communication) working in the same laboratory as Cherrington, found that his observed arterial glucose concentrations were similar to ours when he studied unanesthesized dogs. Therefore, the difference between Cherrington's and our values are probably explained by an effect of the pentobarbital anesthesia.

After 3 days of starvation the arterial concentrations of glucagon fell significantly. Our findings are different from those reported by

Marliss (1970) in man. This investigator found that glucagon levels increase with fasting and then would fall to levels slightly above post-absorptive concentrations. Fisher (1976) found that this initial increment in glucagon concentration was not due to an increase in α-cell secretion but due to decreased clearance of this hormone by the kidneys.

Our data does not explain this unexpected fall in glucagon concentration but certain mechanisms, other than laboratory error, could explain this discrepancy. The absence of an increase in the arterial levels of glucagon may be due to a decrease in its secretion by the pancreatic α-cells or to an increase in renal clearance of this hormone.

Glucagon secretion by the α cell is stimulated by hypoglycemia, decreased FFA concentrations and elevated amino acid levels (primarily glucogenic amino acids) (Luyckx, 1970, Madison, 1968, Unger, 1972). Our dogs experienced a fall in amino acid concentration, an increase in FFA levels and a stable glucose level. Therefore, the absence of the rising secretory stimulus present in fasting man (i.e. fall in blood glucose, the presence of an α cell inhibitor, elevated FFA levels) would lead to a decrease in glucagon levels. This could explain the discrepancy between our findings and those of Marliss. Also a difference in the metabolic clearance of glucagon in dogs fed a high protein diet as compared to man fed a high carbohydrate diet cannot be excluded.

In man the fall of glucagon to postabsorptive levels seen with the progression of a fast is due to a decrease in its secretion by the pancreas (Fisher, 1976). It is also at this point that the level of glucose has been stable for some time while the levels of FFA are elevated and the BCAA have fallen. The fall in gluconeogenic amino acids and the elevation of FFA would influence the secretion rate by a negative feedback mechanism

common to biological systems. The lowered but stable concentrations of glucose would fail to stimulate glucagon secretion since the pancreas probably responds to rate of change rather than absolute levels of glucose. Further study on the effects of fasting on the level of glucagon should be done.

Conclusions

This study is an attempt to simultaneously study the interaction of the kidney, hindleg and the different components of the splanchnic bed in the postabsorptive and briefly fasted states using a newly developed chronic dog model. Arteriovenous whole blood concentration differences and regional blood flow determinations have been obtained across these tissues in unanesthesized fully recovered post-surgical dogs with multiple pre-implanted catheters. The following phenomena have been observed:

1. Arterial concentrations of glucose do not change with a brief fast in dogs fed a high protein carbohydrate poor diet prior to the fast.
2. The arterial concentration of the BCAA fell after a brief fast. This fall was due to a decreased efflux of these amino acids from the splanchnic bed and was accompanied by a fall in the levels of glucagon which suggests a decreased rate of liver proteolysis. This explains the decreased plasma delivery rate of the BCAA observed by Sherwin (1978) in the prolonged fasted state.
3. The fall in the BCAA levels and their efflux from the splanchnic bed parallels the falling arterial level of insulin. Therefore, it is possible that the levels of the BCAA rather than the level of glucose are the main determinants of insulin levels in the dog.
4. The fall in the level of glucagon was accompanied by a fall in the level of the gluconeogenic amino acids, a rise in the levels of FFA and a stable blood glucose concentration. This suggests

that the arterial concentration of glucagon is influenced by FFA and gluconeogenic amino acids in the fasting dog.

5. Alanine was the main gluconeogenic amino acid extracted by the liver and could have been responsible for 8% of the glucose produced by this tissue. In both the postabsorptive and briefly fasted states the non-hepatic splanchnic bed, a tissue not yet studied in fasting man, produced up to 96% of the alanine extracted by the liver. Alanine was also produced by the kidney but unlike man it was not significantly released by the hindleg. The reasons for this difference are not known.
6. Glutamine was the only amino acid significantly released by the hindleg and the main amino acid extracted by the non-hepatic splanchnic bed.
7. The rate of ketogenesis after 3 days of starvation was similar to that of man after a brief fast. The arterial ketone acid concentrations were lower than those reported for man which suggest a higher removal of this fuel by the non-hepatic splanchnic bed as well as muscle.
8. As expected there was a small but significant increase in urinary nitrogen excretion from the second to the third day of a fast suggesting increased protein derived gluconeogenesis. Ammonia excretion by the kidney fell significantly with fasting as previously reported in the literature. Glutamine, however, was neither extracted nor released by this organ.
9. The dog hindleg blood flow can be readily measured with the capacitance plethysmograph as adapted by Aoki.

10. The carbohydrate content of the diet preceeding a short fast could explain many of the findings of this project. Further investigation with a larger number of animals and under different dietary regimens should be performed to clarify and elucidate some of the observations stemming from this project.

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Appendix 1

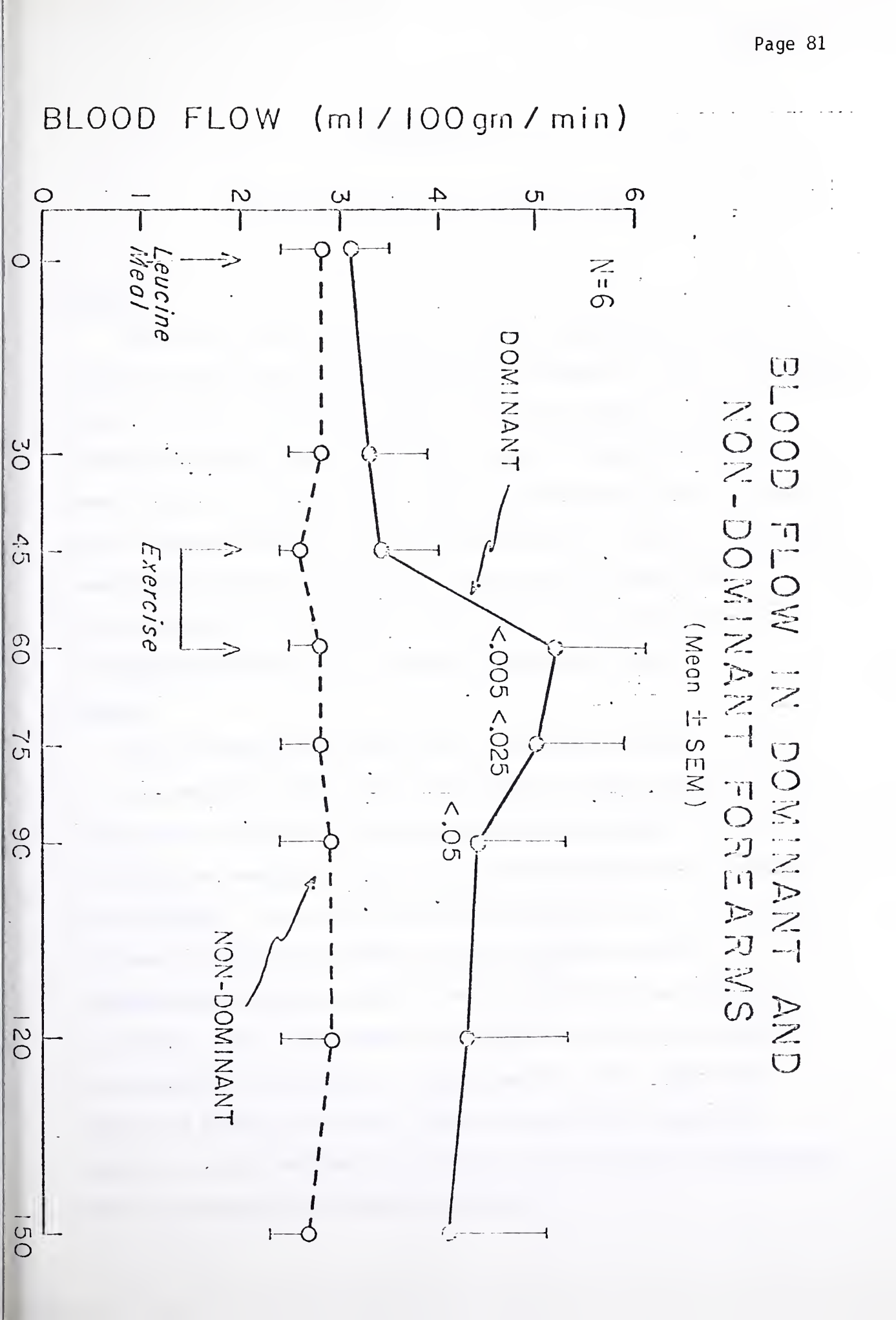
Capacitance Plethysmography
by Thomas T. Aoki, M.D.

With the growing requirement to perform a maximum number of substrate and hormone determinations on a very limited quantity of blood, coupled with the need to measure extremity blood flow, especially that of the forearm, a number of different techniques to quantitate the latter have been considered. Methods investigated have included water or air displacement, strain gauge transducers, dye dilution, and the capacitance plethysmograph. Of these various methods, the capacitance plethysmograph, first described by Figar (Physil. Bohemoslov. 8:275, 1959) and subsequently refined by a number of investigators (Hyman, C. et al., J. Appl. Physiol. 18(5):997, 1963; Wood, J.R. et al., Med and Biol. Engng. 8:59, 1970) most closely approaches the ideal of being non-invasive, accurate, sensitive, reproducible, and simple to operate.

In brief, a copper screen cuff that encircles a substantial portion of forearm muscle serves as one plate of a capacitor while the skin of the forearm serves as the other plate. These two plates are separated by a layer of air filter foam. When a blood pressure cuff is applied to the upper arm and inflated to approximately 40 mm Hg, venous return from but not arterial supply to the forearm is obstructed, and the forearm begins to swell in direct proportion to the amount of blood being pumped into it. Thus one plate of the capacitor, i.e. forearm skin, begins to approach the other plate, the copper screen, and the capacitance of the system increases. This change in capacitance is then converted into a change in voltage by a capacitance plethysmograph (Model 2560, Biocom Inc., Culver City, California). The D.C. voltage changes are then recorded (Weathermeasure EPR-200A). Calibration of the system is accomplished by the inflation of a plastic bladder (Wenger, C.B. et al., J. Appl. Physiol 38(1):58, 1975), applied to the forearm immediately under the copper screen and at the same potential as the forearm, with 5 ml of saline. This change in capacitance is then recorded. The calibration procedure requires less than 10 seconds and is performed immediately prior to measurement of forearm blood flow. Four to five blood flow determinations are performed at each blood sampling time in forearm investigations, and a representative study is attached. In this investigation, basal blood flow in both forearms were measured at -15 and 0 minutes. Subjects were then asked to ingest 14.7 grams of l-leucine. Forty five minutes later, they were asked to squeeze a dynamometer for five seconds followed by a 5 second rest period, with the entire sequence repeated over the next 15 minutes. Note the abrupt change in forearm blood flow in the exercising arm and the lack of change in the resting arm (Mean ± SEM).

To date, forearm blood flows have been measured, using the method described above, in 23 normal subjects, under a variety of experimental conditions, and satisfactory recordings have been obtained in all. In addition, these measurements were quite comparable to those already recorded in the literature with respect to basal flow and exercise.

Currently, baseline calf and toe blood flows are being obtained in both normal subjects and diabetic patients of the Joslin Diabetes Foundation.


BLOOD FLOW (ml / 100 gm / min)
BLOOD FLOW IN DOMINANT AND NON-DOMINANT FOREARMS
(Mean ± SEM)
N=6
DOMINANT
NON-DOMINANT
<.005
<.025
<.05
Leucine meal
Exercise
0
1
2
3
4
5
6
0
30
45
60
75
90
120
150

Appendix 2

Dog Hindleg Composition and Blood Flow

Purpose

The Chronic Dog Project at Joslin Research Laboratories intends to study interorgan metabolism by measuring the movement of fuels and hormones across the liver, intestines, kidney and skeletal muscle. Chronic catheters have been placed in the aorta, portal, hepatic, renal and femoral veins. The dog hindleg, unlike the kidney and liver, is a heterogenous structure composed of skin, bone and muscle. This pilot study was undertaken to determine the relative composition of these tissues in the dog hindleg and the volume covered by the cuff of the capacitance plethysmograph which we use to measure hindleg blood flow.

Methods

Two adult female dogs were used in this study, FA and PA. FA was in a hypermetabolic state running temperatures of 40°C, presumably from a clostridial infection. Both dogs were anesthesized with acepromazine 0.25 mg/pd and nembutol. Blood flow was determined with the capacitance plethysmograph. The cuff of the plethysmograph was placed 1-2 cm above tne patella of the hindleg after the hair had been shaved off. A sphygmomanometer or rubber tube of small circumference was placed on the proximal thigh. Measurements of blood flow in one of the dogs, PA, were determined by occluding the venous outflow with a rubber tube compressing around the proximal thigh and the obtained values were compared to values obtained by occluding the effluent with a sphygmomanometer at pressures of 120 mmHg and 160 mm Hg.

The dogs were sacrificed and their hindlegs were removed at the level of the cephalic femoral artery. The hindleg's relative composition by weight and volume was determined as well as the tissue composition of the area under the capacitance plethysmograph cuff.

Results

The relative tissue composition of the dog hindleg by weight and volume is presented in Table I. The dog hindleg was 8.0% (7-11%)* of the dog's total body weight. The hindleg was 58.5% (53-64%) muscle, 17.75% (17-19%) bone and 23.25% (19-28%) skin by weight. Volumetrically the hindleg was 60.25% (56-66%) muscle, 15% (14-17%) bone and 24.75% (21-27%) skin. The volume under the plethysmograph was 14% (12-18%) of the hindleg and its composition was 75% (69-85%) muscle, 5% (4-6%) bone and 20% (11-25%) skin.

Blood flow values are presented in Table II and III. Fifteen blood flow measurements were done in PA with the sphygmomanometer inflated at 120 mmHg. The blood flow averaged 6.8±1.49 ml/min/100 cc of hindleg$^{\psi}$ which was not significantly different from the 7.30±.73 ml/min/100 cc of hindleg obtained by 7 blood flow calculations at 160 mmHg. The blood flow determined by occluding the venous outflow with a rubber tube averaged 6.19±1.44 ml/100 cc of hindleg/min which was not statistically different from the values obtained with the sphygmomanometer at 120 mmHg or at 160 mmHg. All blood flows were calculated in one session which lasted less than one hour.

Six blood flow measurements were done on FA. FA's blood flows averaged 14.20±7.42 ml/min/100 cc of hindleg at 120 mmHg. Using FA's and PA's total hindleg volumes the hindleg blood flows are calculated to 340.9 ml/min and 90.26 ml/min respectively.

* Mean % (range)

ψ = Mean ± SD_{n-1}, ml/min/100 cc of hindleg

Table I: The Composition of Dog Hindleg

	FA				PA				
NAME	Right	%	Left	%	Right	%	Left	%	Summary: Average
Wt & Temp	30kg	103.8 F			18.3kg	101.0 F			
Hindleg Volume	2401cc		2714cc		1295cc		1096cc		% Hindleg Comp. by Vol.
Muscle	1571	66%	1635	60%	765	59%	616	56%	Muscle 60.25%
Bone	321	13%	434	16%	175	14%	185	17%	Bone 15. %
Skin	510	21%	645	24%	355	27%	295	27%	Skin 24.25%
Plethysmograph Volume	432	18% hindleg	359	13%	160	12%	166	15%	% Plethys. Comp by Vol.
Muscle	325	75%	305	85%	110	69%	118	71%	Muscle 75%
Bone	21	5%	14	4%	10	6%	8	5%	Bone 5%
Skin	85	20%	40	11%	40	25%	40	24%	Skin 20%
Hindleg Weight	2553.1	8.5% body wt	2797.9	9%	1311.4	7%	1996.1	11%	% Hindleg Comp by Wt.
Muscle	1629.1	64%	1693.7	61%	759.3	56%	629.1	53%	Muscle 58.5 %
Bone	487.6	17%	513.3	18%	226.7	17%	226.4	19%	Bone 17.75%
Skin	436.6	19%	590.9	21%	325.4	25%	340.6	28%	Skin 23.25%
Plethysmograph Weight	376.8	15% hindleg	362.5	13%	162.7	12%	168.3	14%	% Plethys. Comp. by Wt.
Muscle	328.4	87%	299	82%	108	66%	119.4	71%	Muscle 76.5 %
Bone	22.8	6%	16.3	5%	11.9	8%	10.8	6%	Bone 6.25%
Skin	25.6	7%	47.2	13%	42.8	26%	38.1	23%	Skin 17.25%

Table II: Hindleg Blood Flow-Plethysmography

PA: Anesthesized with .25mg/pd acepromazine and nembutal.

C_1=28 R_1=4.46cm

C_2=24.5 R_2=3.90cm

Volume under the cuff 192.39cc

#1 Rubber tube to compress venous system

paper speed 16cm/min	Blood Flow
39°	5.62
33°	4.51
36.5°	5.14
37°	5.23
37°	5.23
48°	7.71
46°	7.19
44°	6.71
35°	4.86
33°	4.51
34°	4.68
43°	6.48
36°	5.05
paper speed @ 32.0cm/min	
16°	7.97
15°	7.44
17°	8.49
17°	8.49

$\bar{X} \pm SD_{n-1} = 6.19 \pm 1.44$

#2 Blood pressure cuff to occlude venous outflow

paper speed @ 32.0cm/min		Blood Flow	
120mm	16°	7.97	
	10°	4.90	
	10°	4.90	
	12°	5.90	
	13°	6.41	
	11°	5.40	
	13°	6.41	
	15.5°	7.70	
	10°	4.90	
	16°	7.97	
	17°	8.49	
	12.5°	6.16	
	18°	9.03	$\bar{X}$=6.81±1.49
	14°	6.93	
160mm	16°	7.97	
	14°	6.93	
	15°	7.44	
	12°	5.90	
	16°	7.97	
	15°	7.44	
	15°	7.44	$\bar{X}$=7.30±.71

$\bar{X} = 6.97 \pm 1.29$

Table III: Hindleg Blood Flow

FA: Anesthesized with .25mg/pd acepromazine and nembutol.

Blood Pressure Cuff Occluding Venous Outflow

paper speed 32cm/min

	Blood Flow/100cc
30°	20.6cc
20°	13 cc
36°	25.9cc
23°	15.16cc
25.5°	17.03cc
26°	17.42cc

$\bar{X} \pm SD_{n-1} = 14.20 \pm 8.42$

Conclusions

1) It is assumed that the multiple measurements of hindleg blood flow are independent from each other.

2) -Blood flow determinations with the capacitance plethysmograph can be done by either occluding the venous effluent with a small circumference rubber tube used as a tourniquet or with a sphygmomanometer inflated at 120 mmHg and at 160 mmHg.

3) There is no difference in the blood flow values obtained with the capacitance plethysmograph when the sphygmomanometer is inflated to 120 mmHg versus 160 mmHg.

4) The tissue comprising the dog hindleg as well as that under the capacitance plethysmograph cuff is mainly muscle.

5) PA and FA's blood flow to the dog hindleg differed considerably. This difference between the two dogs can be explained in part by the difference in size between the two dogs as well as the hypermetabolic state under which FA's measurements were done. Big variabilities in blood flow between dog's hindlegs have been reported in the literature (1).

[1] Cronenwett, J. L., Lindenauer, S. M.; Direct measurement of arterio-venous anastomotic blood flow in the septic canine hindlimb. Surgery March 1979. p. 275-282.

Appendix 3

Blood Processing for Analyses

6 ml fresh whole blood
+
6 ml chilled 30% PCA* (use 15 ml centrifuge tube)

1) shake vigorously, parafilm capped
2) spin, discard ppt

4 ml filtrate → Add 1 ml 0.2M Na Acetate Buffer (pH=4.9)

1) Adjust pH to 4.9
2) Bring up to 8 ml final volume c deionized H_2O
3) Freeze@-20°C
4) Label "N-Ketone," pH=4.9

(whole blood glutamine, glutamate, alanines)

4 ml filtrate

1) Label "Ketone"
2) Freeze@-20°C

(lactates, pyruvates, α-ketoglutarate, glycerols)

*30% PCA = 71.4 ml H_2O + 28.6 ml 70% PCA

WB amino acid analyses

Take hematocrit

5 ml whole blood
+
5 ml 10% Sulfosalicylic Acid

1) Vortex for 30" until uniform brown, creamy consistancy
2) Spin in refrigerated centrifuge, discard ppt

Acid filtrate

1) Label: WB-10% SS
2) Freeze@-20°C

Blood NH_3

2 ml fresh whole blood
+
2 ml 10% PCA* (chilled)

1) Shake vigorously, parafilm cap
2) Spin, discard ppt

1) Take 2 ml Acid filtrate adjust pH to 6-8 c 5N, .5N, .05N KOH
2) Bring up to 3 ml final volume c deionized H_2O
3) Label: 10% PCA, pH = 6-8 NH_3
4) Freeze@-20°C

*10% PCA = 108.6 ml H_2O + 11.8 ml 70% PCA

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